AN INTRODUCTION

TO THE STUDY OF THE

DIATOMACEÆ,

BY

FREDERICK WM. MILLS, F.R.M.S.,

AUTHOR OF

PHOTOGRAPHY APPLIED TO THE MICROSCOPE, &c.

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WITH A

BIBLIOGRAPHY,

BY

JULIEN DEBY, F.R.M.S.

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" Tous les ordres des êtres naturels ne forme qu'une seule chaîne, dans laquelle les différentes classes, comme autant d'annimaux, tinnent si étroitment les unes aux autres, qu' il est impossible aux sens, et a l'imagination même, de fixer précisement le point, où qu'une commence ou finit."

LEIBNITZ.

PREFACE.

In making his first acquaintance with the Diatomaceæ the author experienced great difficulty in obtaining concise elementary information. He had to grope his way through a mass of irrelevant literature contained in the reports of Scientific Societies, to purchase many very expensive works, and, with much difficulty, to obtain access to certain rare old volumes.

The study of diatoms is attracting an ever increasing circle of microscopists. By the discovery of fossil diatoms geologists are solving many important and difficult problems. The subject is one of surpassing interest. The field of study is vast, is largely unexplored, and is within the reach of all. Beautiful living specimens abound on every hand, in unpolluted watercourses, in ponds, and in moist places. The great want appears to be a concise introduction both to the diatom and its literature. The author trusts that the following pages may to some extent supply the want. He has collected his information from the highest sources, and has not refrained, where desirable, from using the precise language of eminent writers, whose teaching is better expressed in their own words.

The author acknowledges with gratitude the generous assistance of Mr. Julien Deby, F.R.M.S., who has placed at his disposal the Bibliography appended to this treatise.

F. W. M.

Huddersfield,

April, 1893.

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AN INTRODUCTION TO THE STUDY OF THE DIATOMACEÆ.

CHAPTER I.

Preliminary Remarks.

What are Diatoms? They are a family of Confervoid Algæ (being so minute that a microscope is required to distinguish them), commonly known as brittleworts, and formerly called by Kützing, Bacillariaceæ, from the genus Bacillaria. They are now termed Diatomaceæ from the word Diatoma.

Formerly Diatoms were regarded as belonging to the animal kingdom, but they have long since been definitely classified as Cryptogamia.

The Rev. William Smith, one of our early authorities as to their habits, states:—"The Diatomaceæ inhabit the sea or fresh water; but the species peculiar to the one are never found in a living state in the other locality; though there are some which prefer a medium of a mixed nature, and are only to be met with in water more or less brackish. The latter are often found in great abundance and variety in districts occasionially subject to marine influences, such as marshes in the neighbourhood of the sea, or the deltas of rivers, where, on the occurence of high tides, the freshness of the water is affected by percolation from the adjoining stream, or more directly by the occasional overflow of its banks. Other favourite habitats of the Diatomaceæ are stones of mountain streams or waterfalls, and the shallow pools left by the retiring tide at the mouths of our larger rivers. They are not, however, confined to the localities I have mentioned—they are in

fact, most ubiquitous, and there is hardly a road side ditch, water-trough, or cistern, which will not reward a search and furnish specimens of the tribe."

It is impossible to conceive the extraordinary abundance of Diatoms throughout the world. According to Prof. Ehrenberg, they have exercised an important influence in blocking up harbours and diminishing the depth of channels.

A deposit of mud, chiefly consisting of their siliceous valves, no less than 400 miles long, by 120 miles broad, was found at a depth of between 200 and 400 feet, on the flanks of Victoria Land, in 70° South Lat. Of the thickness of this deposit no conjecture can be formed.

Sir J. D. Hooker's observations upon the Diatomaceæ of the Southern seas show that within the Antartic Circle Diatoms become enclosed in newly formed ice, and are washed up in myriads by the sea on to the "pack" and "bergs," where their presence makes the snow and ice a pale ochreous brown. The same author remarks that the universal presence of this microscopic vegetation throughout the South Polar Ocean is a most important feature, since there is a marked deficiency in this region of higher forms of vegitation. Were it not for Diatoms there would not be food for aquatic animals, nor could the ocean waters be purified from the carbonic acid which animal respiration and decomposition would be continually imparting to them.

Diatoms are found in the digestive organs of fishes, where many rare specimens may often be obtained.

It is worthy of note that singular instances of the preservation of diatoms may be found in guano, into which they have passed from the intestinal canals of the birds of whose accumulated excrement that substance is composed, the birds having received them from shell-fish, to which Diatoms most probably serve as food. They are also found in the fossil state in many places in great abundance.

"As regards the longevity of Diatoms," says the Rev. F. Wolle,* "it may be said that dried specimens can not be revived, but they have been known to survive nearly a quarter of century in their natural element, even though kept for long periods in the dark, and at times frozen in solid ice. Their siliceous covering is almost indestructible, resisting the strongest acids and passing unscathed through very high degrees of heat."

Large deposits of Diatoms in Sweden and Norway are known under the name of *bergmehl*, or mountain-flour; and in times of scarcity the inhabitants mix a quantity of the fine deposit with their flour when making bread. This does not merely increase the bulk of the loaves without adding any nutritive qualities, as some have averred, for the *bergmehl* loses from a quarter to a third of its weight on exposure to a red heat, thus demonstrating that it contains a large quantity of organic matter.

* Diatomaceæ of North America, p. xiii.

CHAPTER II.

The Structure of Diatoms.

All living Diatoms possess a gelatinous envelope, which, owing to its transparency, can only be readily detected by means of adding a colouring matter to the surrounding water. In certain species this gelatinous matter forms a stalk giving the Diatom the appearance of a flower growing on a stem. Inside this outer mass lies the frustule, which is a simple cell, having a firm external coating of silica (Cytioderm). The frustule consists of two symmetrical portions or valves (comparable to those of a bivalve shell), which are in contact at their margins with an intermediate hoop. In the pill-box forms, the top and bottom constitute the valves, the sides are known as the connecting membranes or sutural zones, and when detached are called the hoops. The line of juncture forms a suture, along which the valves may be readily separated.

The outline of frustules is infinitely varied. Almost every conceivable form may be found; triangular, square, and many sided figures oval, boat-shaped, rod-like, circular, globular, saddle-shaped, lunate, are the most frequent shapes. The valves of these beautiful frustules possess markings of varying degrees of fineness, presenting an appearance of dots, groves, ridges, etc., differing with every species. The question whether, in certain species, these markings are perforations, depressions, protruberances or cells within the siliceous wall, has raised in the past, and no doubt will do so in the future, numerous controversies about their actual formation, and the punctate, striate, or other appearances which the microscope presents to us.

Many genera are furnished, on either side of the frustule with a median line, or keel, called the raphé.

The raphé has a central nodule and often at either end it is terminated by other nodules.

The raphé was adopted by Prof. H. L. Smith in his diatomacean classification (which is incorporated in this work) as the primary basis of the families.

Within the frustule is an endochrome, the superficial layer of which constitutes a primordial utricle, which is firmly attached to the silex. "The endochrome of Diatoms consists, as in other plants, of a viscid protoplasm, in which float the granules of colouring matter. In the ordinary condition of the cell these granules are diffused through it with tolerable uniformity, except in the central spot, which is occupied by a nucleus: Round this nucleus they commonly form a ring, from which radiating lines of granules may be seen to diverge into the cell-cavity. Instead of being bright green (as in the Desmidiaceæ), the endochrome is a yellowish brown. The principle colouring substance appears to be a modification of ordinary chlorophyll; it takes a green or greenish-blue tint with sulphuric acid, and often assumes this hue in drying; but with it is combined in greater or less proportion a yellow colouring matter termed *diatomin*, which is very unstable in the light and fades in drying. At certain times, oil globules are observed in the plotoplasm; these seem to represent the starch-granules of the Desmidiaceæ and the oil globules of other protophytes." *

The chemical properties of the endochrome were first investigated by Prof. H. L. Smith, with the micro-spectroscope. Several other authors have fully treated of this subject, especially MM. Kraus, Millardet, Petit.†

In the *Pleurosigma* the oil-globules are four in number; a pair near each end of the diatom. They are not, however, all in the same plane; one globule of each pair being nearer

*The Microscope, by Carpenter, 7th Ed., by W. H. Dallenger, p. 517.
†See also M. Petit's account of endochrome in Bull. Soc. Belg. Micr., V. p. 55, 1880. (A full translation appears in T. R. M. S., iii., p. 680, 1880.)

the observer than the other. Mr. Jabez Hogg states* that the blue-black colour which is assumed by these globules. After the diatom has been treated with hypermosic acid, demonstrates that they consist of oleaginous matter.

Besides the central mass contained in the frustule, the conical cavities at either end of the siliceous shell are seen to be filled with a similar granular substance, and two linear extensions from each of these three masses are developed, closely underlying that part of the shell which is beneath the raphé (the central longditudinal line which is so prominent in the *Pleurosigma*); so that in the side view they appear attached to the right and left edges of the interior of the frustule. This colourless granular substance carries in its centre, near the middle of the diatom, an imperfectly developed nucleus, which is not readily seen, but which may be easily demonstrated by the application of acid. The colourless substance is what, in other diatoms, Schultze shows to be Protoplasm, or vegetable sarcode. It contains numerous small refractive particles; which on the addition of a drop of a one per cent. solution of osmic acid became blue-black and proved to be fat. It is, however, excessively difficult to determine the exact limitations of the protoplasm, on account of the highly refractive character of the siliceous skeleton, and the obstruction presented by the endochrome.

After a short distance, the protoplasm reappears, and is contracted into a considerable mass within the conical terminations of the frustule. Schultze observed in this part of the protoplasm a rapid molecular movement such as is known to occur in the *Closterium*, and further, a current of the granules of the protoplasm along the raphé.

*The Microscope, by Jabez Hogg, 11th Ed., page 424.

CHAPTER III.

The Movement of Diatoms.*

There is nothing more interesting or more obscure than the movement of these microscopical plants.

For the purposes of this chapter Diatomaceæ may be roughly divided into three groups. viz. :—i.—those which live free and apart from each other; ii.—those which live in chains or stalked groups; and iii.—those which live together in large numbers encased by a branching gelatinous envelope. It is, however, only in those species which are free, and in a few of the fixed and chain species, that movements have been observed.

Anyone who gets a handful of duckweed or conferva from a pond, and places some of its droppings under the microscope, is certain to observe some forms of *Navicula*, *Nitzschia*, or *Synedra* exhibiting movements. The motion is of a particular kind, a slow regular advance in a straight line, a little hesitation, then other rectilinear movements, and after a short pause, a return upon nearly the same path by similar movements. It is particularly noticeable that an obstacle in the path does not affect the Diatom, which does not shrink from or avoid foreign bodies in any way, nor is there the slightest evidence that contact with them affects the nature of the movement, as it almost invariably does in the lowest Protozoa. Either the obstacle is pushed on one side by the Diatom, or, should it be too large for this, the Diatom remains pressing against it for as long a space of time as would have been occupied in its forward movement had it

*This Chapter is principally taken from Mr. Ray Lancaster's paper in *The Popular Science Review*, 1866, p. 395.

been unopposed. As compared with the rapid darting evolutions of the Infusoria, the movements of the Diatoms are slow. They have been estimated by the Rev. W. Smith to move about 400 times their own length in three minutes; that is to say, a form $\frac{1}{400}$ of an inch in length moves more than $\frac{1}{2000}$ of an inch in a second.

Referring to the strength and vigour of the movements of Diatoms, Dr. Donkin observed* a species, *Bacillaria cursoria*, discovered by himself, push away *A. arenaria*, a species at least six times its own size. Mr. Barkas states that he has seen them push away particles of foreign matter, and that with the greatest ease, at least one hundred times larger than all the frustules combined; and what is more remarkable still, they not only push the accumulated particles away when they are in their direct line of motion, but if they merely touch them in passing, they drag them with them as though attached by some magnetic attraction or strong cement.

No organ capable of producing these movements of the siliceous frustule are apparent to the observer on his first inspection, and many different hypotheses have been advanced to account for them.

The various explanations offered come under one of three heads, viz.:—

i.—The existence of endosmatic and exosmatic currents;

ii.—The existence of cilia on some part of the frustule; or,

iii.—The existence of a snail-like foot external to the frustule.

Nägeli, in 1849, was the originator of the first of these hypotheses, and his explanation was adopted by Von Siebold and the Rev. W. Smith. Nägeli says:—"The cells have no special organs for these movements. But as in consequence of their nutritive processes they both take in and give out fluid matters, the cells necessarily move when the attraction

Quart. Jour. Mic. Sci., vol. vi., new series, p. 26.

and emission of the fluids is unequally distributed on parts of the surface, and is so active as to overcome the resistance of the water."

Von Siebold demonstrated the existence of currents on the surface of *Naviculæ* by means of indigo, and considered his researches corroborative of Nägeli's hypothesis. He remarked that indigo particles coming in contact with the Diatom remained quite motionless, except along the raphé; here he observed that the granules were carried from the central spot slowly along to the terminal points of the frustule, where they stopped a short time, and were then again carried off in the reverse direction. He found the current occasionally so strong that bodies of some size were set in motion by them. The Rev. W. Smith accepts this theory, for he states in the introduction to his synopsis of the British Diatomaceæ, that that the fluids which are concerned in these actions must enter and be emitted through the minute formina at the extremities of the siliceous valves; and it may easily be conceived that an exceedingly small quantity of water expelled through these minute apertures would be sufficient to produce movement in bodies of so little specific gravity.

We now come to the hypothesis of ciliary action. Ehrenberg was the first to start this explanation with singular confidence and inconsistency, as we shall see that he had already advanced the explanation of a central foot. He gives the following minute details, which have only the most remote foundation in fact, in reference to *Surirella gemma*:— "Long delicate threads projecting where the ribs or transverse markings of the shell joined the ribless lateral portions, and which the creature voluntarily drew in or extended. . . . An animulcule, $\frac{1}{18}$ of a line long, had twenty-four for every two plates, or ninety-six in all." These ciliary processes were stated to be actively vibratile, while the frustule was declared to be perforated with ninety-six apertures for their extrusion. The presence of hair-like processes on all parts of the frustule

is not at all infrequent, and it is not improbable that Ehrenberg was deceived by these. There can be little doubt that these hairs are foreign to the Diatom, and are, in all probability, fungoid growths. Mr. Jabez Hogg tells us* that he has repeatedly satisfied himself that the motive power is derived from cilia arranged around openings at either end (in some also around central openings), which, with the terminal cilia, act as paddles or propellers. However, Mr. Wenham has clearly shown that Mr. Hogg's cilia were mere optical illusions.

Ehrenberg, having abandoned his theory of an expanding, snail-like foot projecting from the central opening in *Navicula*, *Surirella*, *Gemma*, etc., announced the discovery of ciliary processes, situated along the lateral openings of the frustule, capable of extention or retraction at intervals. Kützing, a reliable observer, partially acquiesced in this view. He was of opinion " that the openings or pores in the siliceous valves gave exit to gelatinous substances, analogous with that thrown out during the act of self-division, and which often persists when the act is completed, as observed in *Cæconeis*, *Melosira*, *Cyclotella*, *Fragilaria*, etc."

This writer in the 4th Ed., 1861, of Pritchard's Infusoria considered that, at the time when he wrote, it was not satisfactorily ascertained that minute cilia did not exist along the raphé, causing the currents of indigo particles described by Von Siebold.

Thirdly, and lastly, we have to consider the hypothesis of some form of snail-like foot. Ehrenberg asserted that such a foot was protruded from the central thickened portion of the frustule, known as the umbilicus, which he believed to be a perforation. M. Focke believed in the existance of many such feet, of a temporary character, while Mr. Wenham advanced the following speculations on the cause of the move-

* Journal of the Microscopical Society, 1855, p. 235.

ments, which, from their close approximation to the true state of the case as ascertained by Prof. Schultze, deserve especial attention. He says* :—" If caused by the action of cilia, such extremely rapid impulses would be required to propel the comparatively large body through the water, that surrounding bodies would be jerked away far and wide. A similar effect would be observed if the propulsion were caused by the reaction of a jet of water, which, according to known laws of hydrodynamics, must necessarily be ejected with a rapidity to indicate the existence of the current a long distance astern. I consider there is no ground for assuming the motions of the Diatomaceæ to be due to either of these causes. They are urged forward through a mass of sediment without displacing any other particles than those they immediately come in contact with, and quietly thrust aside heavy obstacles directly in their way with a slow but mechanical power, apparently only to be obtained from an abutment against a solid body. In studying the motions of the Diatomaceæ, I have frequently seen one get into a position such as to become either supported or jammed endways between two obstacles. In this case particles in contact with the sides are carried up and down from the extreme ends with a jerking movement and a strange tendency to adherence, the Diatom seeming unwilling to part with the captured particle. Under these circumstances I have distinctly perceived the undulating movement of an *external membrane;* whether this envelopes the whole surface of the siliceous valves I am not able to determine, nor do I know if the existence of such a membrane has yet been recognized. The movement that I refer to occupies the place *at the junction of the two valves*, and it is caused by the undulation of what is known as the connecting membrane. This will account for the progressive motion of the Diatomaceæ, which is performed in a manner analogous to that of the gasteropoda. The primary cause, however, is different, and not due to any

* Journal of the Microscopical Society, 1856, p. 159.

property of animal vitality, but arises, in my opinion, from the effects of vegetable circulation. I have observed several corpuscles of uniform size travel to and fro, apparently within the membrane which is thus raised in waves by their passage."

The researches of Prof. Max Schultze, of Bonn, were published in December, 1865. He reviews the history of the subject and after some remarks on the external currents in *Rhizosolenia* and *Coscinodiscus*, he proceeds to describe his very minute and careful observations on a marine naviculoid species, the *Pleurosigma angulatum*, which is not infrequently met with on the sea-coast. The *P. angulatum* is to be preferred for examination to the larger *P. balticum*, because the transverse markings on its frustule do not impede to so great an extent the observation of what is going on within. When you have a specimen of *P. angulatum* under the microscope, if crawling, it always has its broad side turned to view, one raphé being uppermost, the other in contact with the glass on which it is placed. Prof. Schultze observed in the protoplasm a rapid molecular movement, such as is well known in the Desmid *Cloisterium*, and further, *a current of the granules of the protoplasm along the raphé*. *P. angulatum* crawls, as do all Diatoms possessing a raphé, along the line of suture. To crawl along, it must have a fixed support. Free swimming movements are never to be observed in this or any other Diatom. Accordingly he invariably found that the raphé was in contact with either the glass slip or the cover glass, or was in opposition to some foreign body of considerable size, even when it was standing on end. Where the Diatom crawled along the smooth surface of the glass cover, Schultze noticed that there was a slight turning of the longitudinal axis; this he connected the with fact that the raphé inclines to opposite sides at either end of the frustule.

Having thus determined that a moving protoplasmic mass was intimately connected with the raphé, and that if there

were an external organ of locomotion, the raphé must prove to be its seat, Schultze's next care was to minutely examine the current of granules which he had discovered in the protoplasm underlying the raphé. If he should find that any of the current protoplasm was flowing *externally*, the motor organ in Diatomaceæ would be discovered. It was impossible to see any such phenomenon by simple inspection. Accordingly, Schultze repeated the experiments of Siebold, and observed the same fact that Siebold and Wenham had seen, namely, that particles of foreign matter stuck to the raphé, as though it were covered with some glutinous material, and were carried slowly along by the action of some current. This he observed in many Diatoms, and found invariably that foreign particles adhered only to the raphé or what corresponded to it. "There is obviously" says Prof. Schultze, "but one explanation; it is clear that there must be a band of protoplasm lying along the raphé, which causes the particles of colouring matter to adhere, and gives rise to a gliding movement. For there is one phenomenon which can be compared with the gliding motion of foreign bodies on the Diatomaceæ, and that is the taking up and casting off of particles of the pseudopedia of the rhizopodo, as observed, for instance, on placing a living Gromia or Miliolina in still water along with powdered carmine. The nature of the adhesion and of the motion is in both cases the same in all respects. And since with Diatoms as unicellular organisms, protoclasm forms the principal part of the cell body, everything suggests that the external movements are referable to the movement of this protoplasm."

Unfortunately, however, as it may seem, the Professor could not see this protoplasm; for although he had clearly witnessed the movement of granular protoplasm along the raphé in the *interior* of the frustule, in no Diatom could he observe it *externally*. When we reflect upon the great difficulties offered by the minuteness of the Diatom, the refraction of the siliceous shell, and the close packing of the

endochrome, we cannot but admire the perseverance and acumen displayed. Having satisfied himself that no external movement of *granular* protoplasm could be detected, after examining *Pleurosigma*, *Surirella*, and many others, our observer came to the only conclusion open to him—that the protoplosm moving on the exterior of the raphé is *hyaline*, and totally free from granules, just as are the pseudopodia of *Gromia* and *Difflugia*, described by him. The perforations in the shell along the raphé are so infinitesimal as to be scarcely perceptible with the microscope, and it is through these holes the protoplasm has to flow. Accordingly, its granules, or those of any visible size, are of necessity kept back within the frustule, whilst the clear hyaline matter flows slowly over the exterior of the raphé—moving with the inner protoplasm, whose flow is more obvious from the presence of the granules suspended in it. Hence the band of protoplasm coating the raphé has the import of a foot, on which the Diatom creeps.

Professor Schultze remarked that there were three types on which the holes for the hyaline protoplasm were arranged. The first presented to us with long open slits on the surface of the frustule, corresponding with the raphé, and is seen in *Navicula viridis*, and its allied forms. The second type had the raphé closed, but with fine openings at either end—this was the case in *Pleurosigma*. The third type was the most common, existing in all those flat Diatomaceæ in which the raphé ran along the narrower edges of the frustule. In these there were numerous openings placed along the elevated ridge at the sides of the frustule corresponding with the raphé. These are seen in *Nitzschia*, *Surirella*, &c.

The movements of the Diatomaceæ other than those of free locomotion are admirably explained by Professor Schultze's observations. The rotatory movements of some of the stalked species are thus readily accounted for, while the connection of the concatenated species which live in a chain consisting of several individuals, united by their long sides or by their angles,

are rendered intelligible. It is by this hyaline protoplasm that they are connected the one with the other. The movements of *Bacillaria paradoxa* and *Bacillaria cursoria*, which have so much puzzled observers, now receive a ready explanation. The *Bacillariæ* are elongated Diatoms attached to one another by their broad sides. They are frequently seen to exhibit the most stange movements. One frustule slides along its neighbour until it is only attached to it by its edge; the next one above performs the same movement, and then the whole colony follows the example, so that they come spread out like a flight of steps; after a short pause the movement is reversed. This curious phenomenon is easily explained by the gliding movement of the hyaline protoplasm of one frustule upon that of its neighbour.

It is now more than ten years since Herr T. W. Engelmann most carefully investigated the preception of light and colour possessed by Diatoms, and other of the lower organisms.* His observations led him to conclude that movement in Diatoms was directly caused by a modification of the interchange of gasses, without apparent addition of a sensation, being intimately connected with the presence of free oxygen, which, if not present, could be produced by light upon these organisms. This being the reason why they are stationary when enveloped in darkness and in want of oxygen. This observer found that after movements had ceased owing to want of light, they might be made to recommence by illuminating the Diatom with the red portion of the spectrum. He found that red between the lines B and C promoted the most active movements, while ultra-red and ultra-violet were ineffective.

* *Vide* Bibliography.

CHAPTER IV.

Classification of the Diatomaceæ, with a Conspectus of the Families and Genera.*

Diatoms are placed in the vegetable kingdom as follows:—

Class Cryptogamia; Sub-class Algæ; Natural order Diatomaceæ, which is divided into three sub-families or tribes, viz:—

I. THE RAPHIDIEÆ, which have a true raphé, at least on one of the valves;

II. THE PSEUDO-RAPHIDIEÆ, having at least on one of the valves a blank space, somewhat like a raphé, they are never furnished with processes, teeth, spines, awns or a true raphé; and

III. CRYPTO-RAPHIDIEÆ, never possessing either a true raphé, or a pseudo-raphé, are generally circular or angular in form, and and often furnished with teeth, spines, etc.

Tribe I.—The Raphidieæ.

[N.B.—s.v.=side view; f.v.=front view.]

Frustules mostly bacillar in s.v.; sometimes broadly oval; always with a distinct raphé and nodules on one or both valves; central nodule rarely wanting or obscure; valves simple or complex; raphé generally prominent in s.v., occasionally in f.v., especially when constricted, with nodules at the constrictions;

Frustules mostly bacillar in s.v.; sometimes broadly oval; without teeth, spines, awns, or processes.

* This classification here given is that of Prof. H. L. Smith, of Geneva, revised by the author in accordance with our present knowledge of the subject. Prof. Smith's conspectus was published in "*The Lens*" in the year 1873, and there are only six copies at present known in existence, as almost the whole edition perished in the great fire at Chicago.

Analysis of the Families.

(The figures refer to the corresponding numbers on the left of each section)

Frustules with valves alike 2
Frustules with valves unlike... 1

1 { Valves cuneate... ... ... *Gomphonemeæ*
  { Valves not cuneate 7

2 { Valves symmetrically divided by the raphé ... 4
  { Valves not so divided... 3

3 { Valves alate, or obliquely striate 4
  { Valves not as above, more or less arcuate or cymbiform ... ... *Cymbelleæ*

4 { Valves with central nodule equally distant from ends ... ... *Naviculeæ*
  { Valves not as above 5

5 { Valves with central nodule wanting ... *Naviculeæ*
  { Valves not as above 6

6 { Valves with central nodule unequally distant from ends ... ... *Gomphonemeæ*
  { Valves not as above 7

7 { Frustules genuflexed, nodule or stauros on one valve, usually on the concave margin (at the constriction), valves rarely broadly oval... *Achnantheæ*
  { All others, valves generally broadly oval, rarely bent ... ... *Cocconideæ*

Analysis of the Genera.

FAMILY I.—*CYMBELLEÆ.*

Frustules sometimes complex; frequently hyaline, inflated, or constructed in f.v.; with central nodules approximate and touching the connecting zone; median line often inflexed. Valves frequently with a tranverse line (stauros); connecting membrane often longitudinally striate or punctuate ... ... *Amphora*
Frustules not as above 1

1 { Frustules free, terminal nodules near the ends, raphé more or less arcuate... ... ... *Cymbella*
Frustules often in gelatinous tubes ... ... *Encyonoma*

FAMILY II.—*NAVICULEÆ.*

Frustules compound, valves with loculi (marginal cells) ... ... *Mastogloia*
Frustules not as above 1

1 { Frustules compound (each with two plates); one (superior) with tranverse ribs the other (inferior) striate, with raphé and nodules ... ... *Stictodesmis*
Frustules not as above 2

2 { Valves with a conspicuous, tranverse smooth siliceous band (stauros) not alate ... ... *Stauroneis*
Valves not as above 3

3 { Valves sigmoid or arcuate; or frustules not alate, or raphé inflected or reflected 10
Valves not as above 4

4 { Valves with a central nodule... 5
Valves without a central nodule, or obscured ... 9

5 { Valves having unique raphé 6
Nodules central and terminal elongated between the branches of a double raphé ... ...*Vanheurchia*

6 { Valves with connecting zone, and straight raphé .. 7
Valves with connecting zone, raphé sigmoid...*Scoliopleura*

7 { Terminal nodules elongated, suture slightly elongated ... ... *Colletonema*
Valves not as above 8

8 { Frustules free ... ... *Navicula*
In gelatinous tubes ... ... *Schizonema*

9 { Valves with nodule median wanting or obscured ... ... *Amphipleura*
Valves with median nodule ... ... *Berkeleya*

10 { Valves symmetrically divided by the raphé, frustules not alate, rarely constricted in f.v. ... *Pleurosigma*
Valves not as above 11

11 { Valves keeled, not symmetrically divided by the raphé, which is arcuate, ends reflexed, and marginal ... ... *Toxonidia*
Valves keeled 12

12 { Slightly constricted in f.v.; valves unevenly divided divided by the keel ... ... *Plagiotrapis*
Much constricted, valves equally divided 13

13 { Elongated nodule at each side of keel ... *Amphipora*
Not as above ... ... *Donkinia*

FAMILY III.—*GOMPHONENIEÆ.*

Frustules curved in f.v.; nodule on concave of valves... ... ... *Rhoikosphenia*
All others ... ... *Gomphonema*

FAMILY IV.—*ACHNANTHEÆ.*

Valve with straight raphé ... ... *Achnanthes*
Valve with sigmoid raphé ... ... *Achnanthidum*

FAMILY V.—*COCCONIDEÆ.*

Valves with marginal cells ... ... *Orthoneis*
Valves not as above 1

1 { Valves not symmetrically divided by the raphé *Anortheis*
{ Valves simple, the superior being without a pseudo-raphé, inferior having raphé and nodules *Cocconeis*

Tribe II.—Pseudo-Raphidieæ.

Frustules generally bacillar in s.v., sometimes broadly oval; or suborbicular—very rarely orbicular; with or without nodules

- always with — a pseudo-raphé (simple line or blank space) on one or both valves; or longitudinal septae or vittæ in f.v.; or valves fusiform, sigmoid, beaked, or alate; or with numerous tranverse ribs, plicæ, costæ, striæ, or rows of granules on one or both valves, rarely regularly radial; costæ sometimes showing in f.v.
- without processes, teeth, spines, awns, or true raphé on the valves; except spines very rarely among the Surirelleæ or Tabellarieæ, when the character is already sufficiently indicated by the above.
- never — angular in s.v.
- rarely — hyaline, unstriate; or much developed in f.v., unless longitudinally septate.

Analysis of the Families.

Frustules compound; rarely or apparently longitudinally septate or vittate; septæ or vittæ showing distinctly in f.v. 1
Frustules not as above, or only seen in s.v. ... 2

1 { Arcuate in f.v. (apparently septate?), valves alike or differing only by a pseudo-nodule at ends of concave valve ... ... *Fragilarieæ*
{ All others ... ... *Tabellarieæ*

2 { Valves circular, sub-circular, very broadly oval, or differently costate 3
{ Valves not as above 4

3 { Valves mostly hyaline, with a few pervious costæ (scalari form), or arcuate f.v., with valves differently costate, rarely septate in f.v. ... *Tabellarieæ*
{ All others ... ... *Surirelleæ*

4 { Valves fusiform, sigmoid, beaked, or one margin more strongly marked than the other ... *Surirelleæ*
{ Valves not as above 5

5 { Valves traversely undulate (undulations conspicuous in f.v.), with transverse shaded bands *Surirelleæ*
{ Valves not as above 6

6 { Frustules in f.v. with beaded margins (especially on one side), without ends of costæ; or carinate, or alate ... ... *Surirelleæ*
{ Frustules not as above 7

7 { Valves pervious or dimidiate, costate or striate, or transversely or irregularly dotted (not carinate or alate ... ... *Fragilarieæ*
{ Valves not as above 8

8 { Fustules with a row of marginal sub-capitate processes, alate or carinate ... ... *Surirelleæ*
Frustules not as above ... ... *Fragilarieæ*

Analysis of the Genera.

FAMILY VI.—*FRAGILARIEÆ*.

Frustules arcuate in f.v.; valves with interrupted transverse striæ or costæ; and one or both valves with pseudo-nodules (blank spaces) at the ends ... ... *Gephyria*
Frustules and valves not as above 1

1 { Frustules more or less arcuate in s.v.; with transverse (often granulate) ribs (canaliculi W. Sm.); which in f.v. frequently cause the margin or submargin to appear beaded, or dentate ... *Epithemia*
Frustules not as above 2

2 { Frustules arcuate in s.v.; not ribbed; valves transversely striate on concave margin, without median line or nodule, and with pseudo-nodules at the ends ... ... *Eunotia*
Frustules not as above 3

3 { Valves with a pseudo-raphé, and transverse rows of granules within square cells (clathrate); central and terminal nodules distinct... *Glyphodesmis*
Valves not as above 4

4 { Valves cruciform; with interrupted transverse striæ (not clathrate), central nodule very distinct; frustules in f.v., with terminal vittæ? ... *Omphalopsis*
Valves not as above 5

5 { Valves arcuate, stiræ fine and granulated, with pseudo-raphé and central nodule; frustules free... *Ceratoneis*
Valves not as above 6

6 { Valves with central and terminal nodules (or blank spaces), latter prominent in f.v.; and pseudo-median line (not always distinct); valves frequently constricted or inflated at the middle; frustules cohereing ... ... *Diadesmis*
Valves not as above 7

7 { Valves with a central (generally transverse) blank space, and a central pseudo-ocellus; or with two or more (few) strong pervious costæ in the middle which are prominent in f.v.; and transverse (generally moniform interrupted) striæ, costæ, or square cellules: and terminal nodules *Plagiogramma*
Valves not as above 8

8 { Frustules cohering; quadrangular in f.v., valves without central nodule; striæ interrupted by a smooth median line, or a blank space; valves inflated or constricted; terminal nodules present, and generally promenient in f.v. ... *Dimeregramma*
Frustules not as above 9

9 { Frustules with a serrated suture; valves without a median line, and with transverse conspicuous rows of perles or dots ... ... *Terebraria*
Frustules not as above 10

10 { Frustules in f.v. narrow, linear; valves lancrolate or inflated; with conspicuous moniliform, (generally somewhat radiate) transverse striæ; and a median line or black space (frequently obscure or wanting); nodules absent ... ... *Raphoneis*
Frustules not as above 11

11 { Frustules in f.v. linear; valve cymbilliform, striæ fine; without pseudo-raphé ... ... *Campylosira*
Frustules not as above 12

12 { Frustules sessile, solitary, or in lines, elongated, linear; slightly cuneate; valve finely striate, constricted at one end, and without median line *Peronia*
Frustules not as above 13

13 { Frustules linear in f.v.; somewhat hyaline, and enlarged at one end; valves striate, without median line or nodule; cuneate, and constricted at one end; united in a stellate or zigzag manner *Asterionella*
Frustules not as above 14

14 { Frustules much elongated; valves with a smooth median line or blank space, sometimes obscure; frequently a central pseudo-nodule; transversely striate, never costate; sometimes slightly cuneate, or bent, sessile, filamentous, or attached end to end ... ... *Synedra*
Frustules not as above 15

15 { Frustules very much elongated, straight or undulate; valves inflated at the middle, slender (awn-like), somewhat irregular punctuate in s.v., without median line, or nodules ... ... *Toxarium*
Fustules not as above... 16

16 { Frustules cuneate; margin smooth; valves hyaline or finely striate, with a median line ...*Licmorphora*
Frustules not as above 17

17 { Valves finely striate, punctuate, or more or less hyaline; never costate; median line wanting or obscure. Frustules narrow in f.v., sometimes inflated (undulate-arcuate) or constricted; margins smooth, cohering, forming a straight (rarely zigzag) filament ... ... *Fragillaria*
Valves and frustules not as above 18

18 { Valves lanceolate with heavy markings, f.v. undulate ... ... *Cymatosira*
Frustules not as above 19

19 Frustules cuneate; valves transversely costate or distinctly granular striate, with a median line ... *Podocystis*
Frustules not as above 20

20 Frustules compound; valves costate or ribbed; ends of costæ prominent, sub-marginal and capitate in f.v.; frustules not cuneate... ... *Denticula*
Frustules not as above 21

21 Valves furmished with ridges; f.v. linear; frustules cohering; filament zigzag ... ... *Diatoma*
Valves not as above 22

22 Valves cuneform forming a spiral filament... *Meridion*

FAMILY VII.—*TABELLARIEÆ.*

Frustules linear, or cuneate, and showing moniliform vittæ (ends of costæ) in f.v.; Valves divided into chambers by transverse ribs (scalariform); outer valve finely pervious striate, without median line ... ... *Climacosphenia*
Frustules not as above 1

1 Frustules curved in f.v.; valves costate, dissimilar; septæ rudimentary ... ... *Entopyla*
Frustules not as above 2

2 Frustules in f.v. with straight vittæ, which are usually alternate; cohering, forming a zigzag filament: valves transversely striate, and inflated at centre and ends, margin often finely punctuate *Tabellaria*
Frustules not as above 3

3 Frustules with straight vittæ, in pairs, and interrupted at the ends and centre in f.v. ... *Diatomella*
Frustules not as above 4

4 Frustules with vittæ in pairs, which are straight or undulate in f.v.; not interrupted or enlarged at ends; cohering, forming a zigzag filament ... *Grammatophora*

Frustules not a above... 5

5 Frustules filamentous; vittæ in f.v. somewhat enlarged at the ends (clavate); valves pervious costate; costæ few, showing in f.v. ... ... *Gomphogramma* (*Tetracyclus*)

Frustules not as above 6

6 Valves without pervious costæ, quite smooth, or *very* finely striate; often with fine median line; frustules hyaline in f.v. 7

Valves and frustules not as above 9

7 Frustules with spines at the angles in f.v. ... *Attheya*

Frustules without spines 8

8 Septæ (or vittæ) in f.v., interrupted, alternate *Tessella*

Septæ not interrupted... ... ... *Striatella*

9 Frustules compound; valves with a median line, and generally blank ends; costate or striate. Septæ in f.v. connected by transverse striæ (latticed); frustules cohering, forming a flat filament ... ... *Rhabdonema*

Valves not as above 10

10 Valves mostly hyaline, with a few pervious costæ (scalariform); linear, orbicular, or inflated *Biblarium*

FAMILY VIII.—*SURIRELLEÆ*.

Frustules compound; valves broadly oval, unlike; one costate, the other sieve-like (cribrose), punctuate; without nodules ... *Campyloneis*

Frustules not as above 1

1 { Valves transversely undulate, undulations conspicuous in f.v. ; striate, and with a few transverse shaded bands ... ... *Cymatopleura*
Valves not as above 2

2 { Frustules in f.v. showing a marginal row of short sub-capitate processes; valves transversely costate (scalariform) ... ... *Clavularia*
Frustules not as above 3

3 { Frustules bent in s.v., margins finely dotted, valves without median line, and with an unequally notched inflation at one end ; cohering in a stellate manner ... ... *Actinella*
Frustules not as above 4

4 { Frustules linear in f.v. ; arcuate, with rounded ends in s.v. ; margins punctuate ; valves finely striate, without median line, terminal nodules on the concave margin. Frustules cohering in a zigzag manner, or in tables ... ... *Desmogonium*
Frustules not as above 5

5 { Frustules (inconspicuously) alate ; valves elliptical or linear ; not cuneate ; parallel (dimidiate, marginal, or wavy) striate ; rarely costate ; median line, if present, simple, and not beaded or conspicuously marked ; valves sometimes bent along the longer axis (apparently) ... .. *Tryblionella*
Frustules not as above 6

6 { Frustules alate (sometimes inconspicuously); valves cuneate, reniform, oval or sub-circular, rarely linear, frequently twisted ; with simple median line, or more or less linear blank space ; centre sometimes blank, or finely dotted ; margins or sub-margins strongly marked (somewhat radiate) ; costæ or plicate (canaliculate, *W. Smith.*) *Surirella*
Valves not alate, or cairnate 7

7 { Not alate 8
Wings imperfectly develeped and reduced to a keel generally having large punctuations 9

8 { Frustules saddle shaped, pseudo-raphé in the form of a cross ... ... *Campylodiscus*
Frustules fusiform, bnt slightly silaceous, furnished with 2 or 3 keels arranged spirally *Cylindrotheca*
Frustules not as above 10

10 { Valves having keels diagonaly arranged ... *Nitzschia*
Keels on both valves on the same side of the frustule ... ... *Hantzchia*

Tribe III.—Crypto-Raphidieæ.

Frustules generally circular, sub-circular, or angular in s.v., more rarely elliptical, oval, or bacilar:
- either — much developed in f.v., and filamentous; *or* processes, teeth, spines *or* awns; *or* more or less hyaline or irregular; *or* transversely septate (or costate) in f.v.
- never — with a central *linear* blank space or true raphé, *on* the valves, except a raphé or pseudo-raphé in Raphidodiscus.

Analysis of the Families.

Frustules cylindrical, or flattened, valves alate (terminated by a calyptre); pointed with a bristle... ... ... *Chætocereæ*
Frustules not as above 1

1 { Frustules with valves dissimilar, or mostly smooth; furnished with awns, horns, (elongated processes), spines, or septæ, which are sometimes absent or imperfect in fossil forms; frequently imperfectly siliceous, valves not ribbed radially, or cellulose *Chætocereæ*
Frustules not as above 2

2 { Frustules imperfectly siliceous, connecting zone more or less turgid ; connected in distant series ; valves angular, with long central spine ... *Chætocereæ*
Frustules not as above 3

3 { Valves with a single marginal, or sub-marginal pseudo-nodule ... ... *Coscinodisceæ*
Valves not as above 4

4 { Valves lunate, not transversly costate or septate ... *Coscinodisceæ*
Valves not as above 5

5 { Valves somewhat hisped ; with sinuato-reticulate lines (not rayed) ... ... *Coscindisceæ*
Valves not as above 6

6 { Valves circular or angular, not much developed in f.v., with (obscurely) reticulate centre, and conspicuous, pore-like punctæ ... ... *Cosinodisceæ*
Valves not as above 7

7 { Valves alike, smooth (hyaline), with radiating lines (linear rays not terminating in a spine) ; rays definite (few) ... ... *Asterolampreæ*
Valves not as above 8

8 { Frustules cuneate in f.v., or with decided ocelli, or processes, or tubercules, generally few and prominent in f.v. (not spines alone) 11
Frustules not as above 10

9 { Frustules cohering ; generally much developed in f.v., and cylindrical ; firmly siliceous ; valves rarely hyaline, unlike or elliptical ; without median line ; sometimes apiculate, or conical, or with a peculiar central nodule (spine) ; radiate punctuate, or cellulate ; and frequently with marginal or sub-marginal spines. Frustules cohering by smooth sutural lines, or by marginal spines or teeth, or by a central spine ... *Melosireæ*
Frustules not as above 12

10 { Frustules transversely septate, or costate ; cuneate, angular, or sub-angular 11
Frustules not as above 9

11 { Frustules little developed in f.v., (free), rarely angular, neither lunate nor cuneate ... *Eupodisceæ*
All others, filamentous, generally much developed in f.v. ... ... *Biddulphieæ*

12 { Valve disk more or less undulate, divided into regular compartments, usually alternately light and dark; mostly with marginal or sub-marginal spines or teeth ... ... *Heliopeleæ*
Valves not as above 13

13 { Valves hyaline with umbiblical lines *Asterolampreæ*
Valves not as above 14

14 { Valves with definite, irregular, flexuose, or bifurcate rays; not hispid, nor with marginal spines ... *Asterolampreæ*
Valves not as above 15

15 { Valves hyaline, rays definite, not reaching margin *Asterolampreæ*
Valves not as above 16

16 { Valves with spathulate, cordate, or deltoid rays, their bases frequently forming a hyaline central area ... ... *Asterolampreæ*
Valves not as above 17

17 { Valves with large marginal spaces, which are neither circular nor hexagonal ... *Asterolampreæ*
All others, f.v. angular oval, circular or lunate ... *Coscinodisceæ*

Analysis of the Genera.

FAMILY IX.—*CHÆTOCEREÆ.*

Frustules annulate, cohering, elongate, ends alike, calyptriform; tipped with a spine or mucro; often imperfectly siliceous ... ... *Rhzosolenia*

Frustules not as above 1

1 — Frustules with two or more horns (elongated processes, not spines simply); valves frequently alike 2

Frustules smooth, or with spines, bristles, or horns 3

2 — Frustules compressed, the sutural portion narrow; horns frequently branching or bifurate; sometimes mucronate; valves and horns sometimes with short scattered spines; horns sometimes obtuse, short obtuse (mammæ) ... ... *Dicladia*

Frustules elongated; horns mucronate; valves dissimilar; generally one horn (or process) on one valve, two on the other ... ... *Syringidum*

3 — Only one valve spined; spine frequently long and sometimes branching ... ... *Syndendrium*

Frustules not as above 4

4 — Valves angular; spine central; sutural portion more or less turgid (imperfectly siliceous) with a radiate series of dots; frustules connected in a distant series ... ... *Ditylium*

Valves not as above 5

Spines (setæ ?) marginal, on both valves; valves dissimilar and mostly hyaline ... ... *Hercotheca*

5 — All other forms, valves frequently awned, or with minute scattered spines, or dissimilar, hyaline or imperfectly siliceous; or frustules compound; in fossil forms awns often absent, leaving valves entirely smooth ... ... *Chætoceros*

FAMILY X.—*MELOSIREÆ.*

Frustules apiculate (drawn out at the extremities or margins to a point) 1
Frustules not as above 2

1 { Frustules cylindrical; apiculate in f.v.; valves unlike ... ... *Pyxilla*
Frustules not cylindrical; apiculate in s.v.; valves alike ... ... *Peponia*

2 { Valves with a central spine, or coronal or scattered spines; not ribbed; frustules cohering by the spines... ... ... *Stephanopyxis*
Valves not as above 3

3 { Frustules cylindrical, with somewhat large marginal teeth, and peculiar central spine ... *Syndetocystis*
Frustules not as above 4

4 { Valves elliptical or constricted, marginal spines or teeth, and peculiar central nodule ... *Rutilaria*
Valves not as above 5

5 { Frustules cylindrical, ends constricted and finally expanded into a connecting nodule *Strangulonema*
Frustules not as above 6

6 { Frustules cylindrical, with border of much elongated cells at junction of margins in f.v. ... *Skeletonema*
Frustules not as above 7

7 { Valves circular, with curved marginal rays, and minute marginal teeth ... ...*Discosira—Melosira*
Valves not as above 8

8 { Valves dissimilar, somewhat conical or inflated in f.v.; with radiating lines or ribs, not branching nor bifurcate at extremities; apex truncated; usually spinous; interspaces punctate *Stephanogonia*
Frustules not as above 9

9 { Frustules marked with spiral or crossed bands in f.v. ... ... *Liparogyra*
All others ... ... *Melosira*

FAMILY XI.—*BIDDULPHIEÆ.*

Frustules with one neck-like process; generally oblique; cohering, irregular, valves unlike *Isthmia*
Frustules not as above 1

1 { Frustules transversely costate in f.v.; costæ more or less capitate, resembling music notes; valves tranversely costate; without spines or median line ... ... *Terpsinœ*
Frustules not as above 2

2 { Frustules transversely costate or scalariform; costæ (septæ) showing in f.v., not capitate, valves often lunate, connecting zone hyaline or striate *Anaulus*
Frustules not as above 3

3 { Frustules generally hyaline or slightly silaceous, forming a filament straight or curved 4
Frustules not as above 6

4 { Frustules with short processes; valves devoid of spines... 5
Frustules furnished with stout spines *Lithodesmium*

5 { Frustules mostly hyaline or imperfectly sillceous; forming filaments;
Filament curved, valve having 2 processes... *Eucampia*
Filament straight, valve having 3-4 processes *Bellerochea*
Processes not as above 6

6 { Processes often elongated, generally straight on the outer margin in f.v., and terminated with a spine or mucro, which is sometimes obscured ... *Hemiaulus*
Frustules not as above ... ... *Biddulphia*

FAMILY XII.—*EUPODISCEÆ.*

Valves with plum-rose rays or dots about the flat mastoid processes (or ocelli); rarely obscure; somewhat with a sub-quadrate central portion, or a radiant cellulation interrupted by a linear series terminating in the ocelli ... ... *Auliscus*

Valves not as above 1

1 — Valves with decided ribs, rays, or furrows, connecting the (usual large) processes or tubercles *Aulacodiscus*

Valves not as above 2

2 — Valves circular, or oval; with ocelli, or pseudo-openings, in compartments ... *Craspedoporus*

Valves discoid with a central thickening or obscure nodule, and an interrupted raphé terminated by minute spines, or spiniform nodules somewhat within the margin of the disk; central portion of disk naviculoid, depressed, its ends terminating at the spines. Striæ radiate moniliform, extending from raphé to the margin of the valve ... ... *Raphidodiscus*

Valves not as above 3

3 — Valves circular, with radiating series of minute punctæ, and marginal tubercles ... ... *Perithyra*

Marginal tubercles smaller, valves radiate granulate, circular or oval ... ... *Cespodiscus*

All others, ocelli (tubercles, or processes) generally quite large, and few, usually sub-marginal; cellules or granules, rarely radial, or minute *Eupodiscus*

FAMILY XIII.—*HELIOPELTEÆ.*

Valves with marginal spines obsolete, or if present, few, and in alternate compartments ...*Actinoptychus*

Valves not as above 1

1 { Valves with a hyaline (stellate) umbilicus, with marginal spines or teeth, connected by a radial rib .. .. *Halionyx*
1 { Valves with numerous marginal spines or teeth; and a hyaline umbilicus; often hyaline spaces at the base (angles) of each compartment *Heliopelta*

FAMILY XIV.—*ASTEROLAMPREÆ.*

Valves hyaline, angular or circular, with straight rays or ribs not expanded at margin or centre, and not reaching the margin ... ...*Liostephania*
Valves not as above 1

1 { Disk radiate-punctate, cellulate or granulate; with several well-defined linear blank spaces (ribs), from the margin inwards; centre granulate, not stellate .. ..*Actinodiscus*
1 { Valves not as above 2

2 { Valves inflated, hyaline, or punctate, centre sometimes stellate; rays linear, more or less bifurcating and somewhat irregular; interspaces blank, or with curved or sinuose lines ... *Cladogramma*
2 { Valves not as above 3

3 { Valves hyaline; with a broad margin divided by simple rays; centre hyaline, or granulate, reticulate, or minutely punctate ... ... *Mastogonia*
3 { All others ... . *Asterolampra*

FAMILY XV.—*COSCINODISCEÆ.*

Disk with a circle of large marginal or intra-marginal cellules; and radiate, or scattered cellules or punctate ... ... *Heterodictyon*
Valves not as above 1

1 { Disk with an interior ring of cellules separating the centre from the broad marginal rim; cellulation of centre, curved or spiral ... ...*Brightwellia*
Valves not as above 2

2 { Disk very complex, or conical in f.v., with a conspicuous, central, pseudo-opening ... *Porodiscus*
Valves not as above 3

3 { Disk cellulose, large, with a broad border of a different structure, separated by a well-defined margin ... ... *Craspedodiscus*
Valves not as above 4

4 { Disk hyaline, with distinct umbilicus, and (very) finely marked; having raged or decussating lines *Hyalodiscus*
Probably often valves of ... ...*Podosira—Meliosira*
Valves not as above 5

5 { Disk cellulose, with narrow (somewhat dentate) rim; connecting zone cellulose ... *Endictya—Meliosira*
Valves not as above 6

6 { Disk without marginal spines, teeth, or pseudo-nodule; usually of small or medium size; and generally with an outer ring-like portion either smooth or striate; centre often bullate, smooth, or granulate: granules equal, scattered, or rayed, *or* disk hyaline (or finely punctuate), with strong linear straight lines... ... . *Cyclotella*
Valves not as above 7

7 { Frustules complex? Disk circular, generally with a marginal or submarginal pseudo-nodule (sometimes absent); frequently with minute marginal spines or teeth; and with a single or double series of radiating dots (in this form ⋖) or punctate often subulate (!) blank spaces ... ... *Actinocyclus*
Frustules not as above 8

8 { Frustules cuneate in f.v., lunate in s.v. 9
Frustules not as above 11

9 { Valves cellulose, centre blank, margin veined *Hemidiscus*
Valves not as above 10

10 { Valves with indistinct umbilicus, finely punctate with radiating lines, dorsal and neutral margins with minute teeth or spines ... ... *Pameria*
All others dorsal margin without spines, ventral frequently with small pseudo-nodule ... *Euodia*

11 { Disk with a radiating series of small equal or sub-equal granules and generally with a granular umbilicus or centre; marginal teeth or spines rarely absent... ... ... *Stephanodiscus*
Valves not as above 12

12 { Valves circular much inflated. Frustules in f.v. with the longitudinal axis much longer than the transverse; not ribbed, nor cellulose; sutural portion ("connecting zone") narrow; sometimes minute marginal teeth ... ... *Pyxidicula*
Valves not as above 13

13 { Valves elliptic, circular, or sub-circular; with a prickly aspect (hispid) often with minute spines, and with sinuato-reticulate rays or lines... *Liradiscus*
Valves not as above 14

14 { Disc circular or angular, with conspicuous punctæ, and divided into more or less plicate compartments, often obscured, by radiating, often dichotomizing, lines or blank spaces; centre sometimes bullate, or more or less distinctly reticulate ... ... *Stictodiscus*
Valves not as above 15

15 { Frustules compound. Disk circular, with numerous strong. straight, radial ribs, and a hyaline centre, ribs connected by concentric lines, or rows of gemmaceous granules without spines or teeth ... *Arachnoiducus*
Frustules not as above 16

16 { Disk with a circle of well-defined marginal or inter-marginal (subulate) spines; cellules in parallel rows ... ... *Systephania*
Cellules not in parallel rows, valves of ... ... *Creswelliia-Stephanopyxis*
Valves not as above 17

17 { Disk very convex, and strongly celulose, without marginal teeth or spines ... ... *Dictyopyxis*
Valves not as above 18

18 { Disk without rays; frequently hyaline; and with scattered spines or spines ... ... *Xanthiopyxis*
All others, without strong linear rays, or large spines, or teeth ... ... *Coscinodiscus*

Dr. A. M. Edwards doubts the existence of true species, or even genera, among the Diatomaceæ.* At all events he claims to have established that all the various species of *Schizonema* and *Homœocladia* are but forms of two species, while both these genera must be united with *Nitzschia*. Again, there are no good characters to distinguish *Schizonema* from *Navicula;* and the twenty-four species of *Micromega* can all be grouped under *Navicula fœtida*. Dr. Edward's arguments are based on the fact that he finds specimens of the alleged different species and genera "in the same tube!"

* Amer. Mon. Micr. Journ., xiii (1892) p. 212—6.

CHAPTER V.

Modes of Reproduction.

Much has been written but little is known of the means by which the Diatomaceæ reproduce their species. It seems to be generally acknowledged that they have three modes of multiplication :—by simple division, auxospores, and by a kind of conjugation which is regarded by some as sexual; the three modes, however, pass gradually one into the other.

Simple division commences with the bipartition of the nucleus, and the division of the internal membrane takes place at the same time, as is seen in higher grades of plant life. When the nucleus is about to divide, the two valves of the frustule separate, the contents dividing into two daughter-cells, and new siliceous valves are formed within the old ones, being therefore smaller. This has led Pfitzer to imagine that when diatoms have reached their smallest possible dimensions by repeated binary division, the process of conjugation takes place between them, resulting in the formation of an *auxospore*, capable of reproducing two sporangial frustules of considerably larger size, which would again give rise, by fission, to a new series of diminishing frustules. Dr. Van Heurck states it as a fact, worth noting, that the protoplasm of the primordial utricle generally moves to the centre of the siliceous envelope, probably at the commencement of duplication of that utricle, and again after the phenomenon is terminated, drawing with it the endochrome, and that movements of the coloured matter differ according to the nature of the different genera and families. "But," states Dr. Dallenger, "binary subdivision cannot take place in genera with unequal valves, as it is universally acknowledged that the two new valves which are

formed in the process of binary subdivision must stereotype themselves on the old valves; and for this reason this process cannot take place in those genera in which axes cross one another, like *Campylodiscus*, or in those in which the two valves, although equal, yet constantly unite in such a way that the similar parts alternate with one another, as may be seen in *Asterolampra*."

In some genera undergoing conjugation, a *zygosperm* is produced as the result of the coalescence of the protoplasmic contents of two different individuals. The conjugating frustules are side by side enveloped in a gelatinous mass, and upon the separation of the two valves the contents of each come in contact and unite in a single zygosperm. In other cases two zygosperms result from the conjugation of a pair of cells. As the protoplasm emerges from the cells it puts out two protuberances, these meet in pairs, and the whole contents of the pair of mother-cells finally pass into the two zygosperms, which complete their development in precisely the same way as the auxospores.

De Bary thus summarises* the four modes in which the Diatomaceæ are reproduced by means of auxospores or zygosperms:—

(i.) Two products of conjugation are formed by the union of the contents of two distinct individuals;

(ii.) A similar process results in the formation of a single product of the same nature;

(iii.) A single act of conjugation (production of auxospores) takes place between two portions of the contents of the same individual; and

(iv.) Two such acts of conjugation take place simultaneously between different portions of the contents of the same individual. In all cases the formation of a new individual is completed by the simple division of the product of union

* Bot. Zeit., 1858, Supplement, p. 61; The above translation is taken from Bennett and Murray's Cryptogamic Botany, London, 1889.

(auxospore or zygosperm) into two symmetrical halves, with or without the intervention of a period of rest.

It may be worthy of note that the tube, frond, stipes, cushion, or mucus pellicle, by which diatoms attach themselves to various objects, such as algæ, bridges, bottoms of boats, etc., is called the Thallus. M. C. Dr. Lanzi observed* in a gathering of *Epithemia ventricosa* taken in Rome, that some portions of the pellicle were composed of a great quantity of round granular corpuscles of a greenish yellow colour. Most of these corpuscles appeared to be similar to those contained in the frustules of the *Epithemia*, and imbedded in a hyaline plasm. At another time he found in a gathering *Cymbella* in a state of reproduction, and was again able to see the round corpuscles, which were small and coloured like the endochrome. They were contained in the thallus, and resembled those in the frustules. On following them through their phases of development he, by repeated observations, ascertained that while increasing in breadth, they preserved their circular form; that afterwards they commenced to elongate, so as to acquire the lunate and naviculoid outline of the mature frustule.

Of these growing forms, some remained attached to the thallus, and others became free. Their number was considerable; and he was easily convinced that they were the result of a new kind of generation. The disparity in size was so considerable, that it would have been absurd to suppose that they had been produced by fissiparity.

Dr. Lanzi was also able to report of other similar acts observed in *Navicula ambigua*, *Nitzschia minutissima*, and *Amphora ovalis*; but in order to avoid repetition confined himself to *Gomphonema olivaceum* only, in which he had followed the series of transformations until the frustule containing the germs became changed into a sporangial cell, and

* Annales de la Société Belge de Microscopie, Vol. IV.

the thallus became charged with germs and frustules in various stages of development. When this cycle was completed, the thallus contained three different forms :—(i.) the sessile sphenelloid form, (ii.) the pedunculate (either simple or dichotomous), and (iii.) the perfect or free form.

Mr. F. Kitton states* that the presence of the "thallus" is by no means uncommon, and he remarks that the reproduction of the Diatomaceæ has not received that amount of attention the subject deserved. Their increase by self-division was the method first observed, more careful observations led to detection of the production of sporangeal frustules, or the formation of a sporangium by a single frustule.

* Transactions of the Royal Microscopical Society, 1879 (old series, vol. ii.) page 38—40.

CHAPTER VI.

Collecting Diatoms.

The diatomist has not far to turn in quest of objects for collection and preservation. As has already been observed in the first chapter, this class of microscopic life is almost universal. It may be met with in fresh, salt, or brackish water in a living state, and large beds of frustules in a fossil state exist; as for instance at Dolgelly. Our mountain streams, lakes, ponds, and ditches contain vast numbers of specimens, which may be collected all the year round. Oyster beds abound with them, and the stomach of almost every fish will yield a good supply.

"Their living masses," says the Rev. W. Smith, "present themselves as coloured fringes attached to larger plants, or forming a covering to stones or rocks in cushion-like tufts (or spread over the surface as delicate velvet), or depositing themselves as a filmy stratum on mud, or intermixed with the scum of living or decayed vegetation floating on the surface of the water. Their colour is usually a yellowish-brown of a greater or less intensity, varying from a light chestnut in individual specimens to a shade almost approaching black in the aggregated masses. Their presence may often be detected without the aid of a microscope, by the absence in many species, of the fibrous tenacity which distinguishes other plants. When removed from their natural position they become distributed through the water, and are held in suspension by it, only subsiding after some little time has elapsed."

The Diatomaceæ have been divided into two classes, by Herr Johann Nave, viz.:—the free species, or those which have an independent existence; and the stipate, or such as

are attached to other objects, generally Algæ, by means of a stalk. There is however a third division comprising the frondose species, or those in which numerous individual frustules are enclosed, held together by a gelatinous mass, and in appearance not unlike sea-weed.

The free growing species are found entangled with algæ and mosses, or below the surface of the water, wherever the soil, stone, or a fallen leaf is stained with a yellowish brown hue. In rapid streams and mountain torrents they become scattered and numbers of them remain suspended in the foam and may be easily gathered. Also when the sun is shining they liberate oxygen and rise to the surface with the gas. Whenever these minute bubbles are seen to be coloured brown, it may be taken as a sure sign that Diatoms are present.

Certain stipate Diatoms are exactly similar to others of the free kind so far as their frustules are concerned, and their stipate existence forms a convenient basis for distinguishing the families. As examples of the close approximation in the form of the frustule may be mentioned, the *Cymbella*, and *Cocconema; Sphenella*, and *Gomphonema*, which are respectively separated solely on account of this difference.

Stipate Diatoms may be found adhering to the larger algæ and similar water plants, tinted with a reddish brown.

When collecting, a number of phials with wide mouths, furnished with corks, should be carried.* The mouth of the bottle being closed with the thumb it is brought close over the coveted colony in the water, and on removing the thumb, the water and Diatoms will rush in. A spoon is frequently used in removing layers of Diatoms from sand at the bottom of pools and rivers. If the masses of Diatoms are entangled amongst algæ, they may be detached and collected

*These can be obtained in a case from Mr. C. Baker, 244, High Holborn, London, W.C.

by means of muslin nets affixed to a rod. Telescopic rods, like Japanese fishing-rods can be procured for carrying the net or a bottle, and are often useful in obtaining specimens which would be otherwise beyond reach.

Diatomaceæ are, as has already been stated, found in fossil form in great numbers, seldom however containing a great variety of species. The mass may sometimes be disintegrated by placing lumps of it in a test-tube, covering them with *Liquor potassæ*, boiling for a short time until the whole breaks up into a sort of mud, which should instantly be poured into a quantity of distilled water and well washed.

CHAPTER VII.

Mounting Diatoms.

There are numerous methods of mounting diatoms but only the most simple and efficient will be here enumerated.

To Mount *in situ*.

Mr. Rylands' method as given in Mr. Davies' book* is as follows:—"Take a shallow ring of asphalt or black varnish (which must be at least three weeks old), and on the cell, whilst revolving, add a ring of benzole and gold-size mixed in equal proportions. In a minute or two pure distilled water† is put in the cell until the surface is slightly convex. The object having been already floated on to the cover (the vessel used for this purpose being an ordinary Indian-ink pallet), is now inverted and laid carefully upon the water in the cell. By these means the object may be laid down without being removed. The superfluous moisture must not be ejected by pressure, but a wetted camel-hair pencil, the size made in an ordinary quill, being partially dried by drawing through the lips, must be used repeatedly to absorb it. The pencil will draw by capillary attraction as it is very slowly turned round. When the cover comes in contact with the benzole and gold-size ring, there is no longer any fear of the object being removed, and a slight pressure with the end of the cedar stick of the pencil will render the adhesion complete, and cement the cover closely and firmly to the cell. When dry, an outer ring of asphalt makes the mounting neat and complete."

* The Preparation and Mounting of Microscopic Objects, new edition. London, W. H. Allen & Co., 1890.

† One-tenth of pure spirit should be added. F.W.M.

Although Mr. Rylands says that he has had no failures, the author finds that unless a very small quantity of a solution of bichloride of mercury, one in a thousand, be added to the water, the water, containing more or less organic matter, will speedily lead to decomposition of the specimens. Even the gelatinous covering of the Diatoms is sufficient to cause decomposition of the preparation, and soon render an otherwise permanent specimen worthless. The solution of mercury must be handled with care, as it is a very strong poison.

Method of Preparing Frustules for Mounting Dry, or in a Medium.

The Diatoms having been separated from the algæ or other matter with which they may be entangled, by agitating the water in which they have been taken, and, if necessary, by the aid of a camel-hair pencil, they are placed in a glass beaker of about eight ounces capacity. The larger and heavier frustules will gradually sink to the bottom, when the rest of the water containing the lighter ones should be decanted off and placed in a similar beaker for them to subside. This may be repeated several times until all the Diatoms have fallen to the bottoms of the several beakers. The decanting is best done with a pipette. Each of the deposits in the beakers should be examined by a power of a one inch, or a two-thirds object glass under the microscope, and by this means the student will learn how to obtain the particular gatherings of which he may be in quest. If on examination any deposit appears to be contaminated with dirt, water and a few drops of liquid ammonia may be added, the whole thoroughly agitated for some minutes with a glass rod, the Diatoms allowed to again subside and the water drawn off with a pipette. It may be necessary to repeat this operation several times, before the Diatoms are free from dirt. When this has been accomplished place the

residue in a large test tube, and add drop by drop nitric acid and boil over a spirit lamp, shaking the tube during this process until all traces of organic matter have been eliminated. Some gatherings require to be boiled in pure nitric acid until the frustules become dark in colour, and then by adding a cold saturated solution of chlorate of potash, drop by drop, to avoid an explosion, until the gathering loses its dark hue.

The Diatoms must now be thoroughly freed from all trace of acid or potash by thoroughly washing in several changes of water, being violently agitated in each, and of course allowed to settle between each decanting, when they will be ready for mounting.

Mounting Dry.

A small drop of water containing the frustules is transferred to a cover glass and allowed to dry, after which it is placed on a small platinum disk attached to a handle, and held over the flame of a spirit lamp, until the cover assumes a dull red heat. By these means the Diatoms will be affixed to the cover. The cover can be mounted over a shallow cell either dry or filled with any medium.

Mounting in Media.

Canada balsam, monobromide of napthaline or any other medium may be used to mount Diatoms by placing the same in the shallow cell.

Mr. E. M. Nelson writes in the Journal of the Qukett Microscopical Club * describing how to prepare the Rev. Father Thompson's high refractive medium, he says :— "Take *flowers of sulphur*, *bromine*, and *arsenious acid* in the proportions of 8, 10 and 12 respectively by weight. Dissolve

Series II., vol. v., p. 123 (July, 1892, No. 31).

the sulphur in the bromine with gentle heat in a thinnish test tube about six inches long. Over a small Bunsen jet add small portions of the arsenious acid, boil and let the condensed vapours of the mixture cool and fall down the sides again. Be very careful that these do not escape. If none of these have escaped, the proportions given will be correct, but, if they do escape, probably a spot more bromine will have to be added to keep the mixture clear.

"No mechanical directions can be given beyond these. Success is very much like that of a cook in his preparations. When made the mixture should be about the consistency of toffy and much the same in appearance. It should be handled with a piece of platinum wire. The more arsenic the better, and a grain or two of the metal itself may be coaxed in towards the end so long as the mixture remains clear. If properly made this will last, so far as I know, for ever."

Prepared frustules may have their "striæ," or other markings, being hollow, impregnated with mercurous sulphide or other matter.

Mr. C. Haughton Gill described his method of charging the markings of Diatoms in a communication made to the Royal Microscopical Society, 19th March, 1890.* He described several processes, but we shall refer only to two of them. Firstly the

Mercurous Sulphide Method.

For Diatoms not having excessively fine markings this method is the best. It is as follows:—A cold saturated solution of mercur*ous* nitrate (*sub*-nitrate of mercury) is added to an equal bulk of distilled water. The Diatoms together with a drop of metallic mercury, are added to this solution, which should be placed in a test tube and tightly corked.

**Vide* J. R. M. S., 1890. p. 423, also 1891, p. 442.

The longer the Diatoms remain immersed in the liquid the better, weeks being preferable to days.

Before removing them agitate the tube, then draw off the valves suspended in the liquid by means of a pipette, leaving behind any crystals of basic sub-nitrate of mercury which may have formed. Place the Diatoms in a small test tube, and allow them to settle, finally drawing off as much fluid as possible, first with a pipette, and lastly with thin strips of filter paper. When almost dry add to the Diatoms a few drops of a strong solution of ammonium sulphide, which has been recently prepared and which is practically free from dissolved sulphur (*i.e.* not yellow), then shake them up in the test tube, which must be filled up with water and corked and allowed to stand for some hours.

The Diatoms must be thoroughly washed in several changes of water, each being decanted after the valves have fallen to the bottom, as described in the first part of this chapter. The Diatoms, which will now be charged with a black amorphous precipitate, are ready for mounting.

The second method of charging is:—

The Silver Nitrate Method.

A strong solution of silver nitrate (100 grs. to 1 oz. of water) may be substituted for the mercurous nitrate, the process being the same as already described. It is however only suitable to those Diatoms having the finest markings.

Whatever method has been employed to mount the Diatoms, the slide should be finished with a ring of asphalt, and when this has become hard a ring of gold size. The older the size the better.

The cell-making and final ringing will be done with the aid of an ordinary turn-table. Finally label the slide neatly.

It is a very good plan to add a ring of coloured cement to designate the mounting medium. The following are those used by the author:

Dry	...Black cement.
Canada Balsam ...	...White ,,
Balsam and Bensole...	..White and red cement.
Bromoiodide of Napthaline...	Green cement.
Thompson's Medium	...Red ,,

In closing this chapter the author advises the student to make several slides of each of his gatherings, at least one *in situ* and another in balsam.

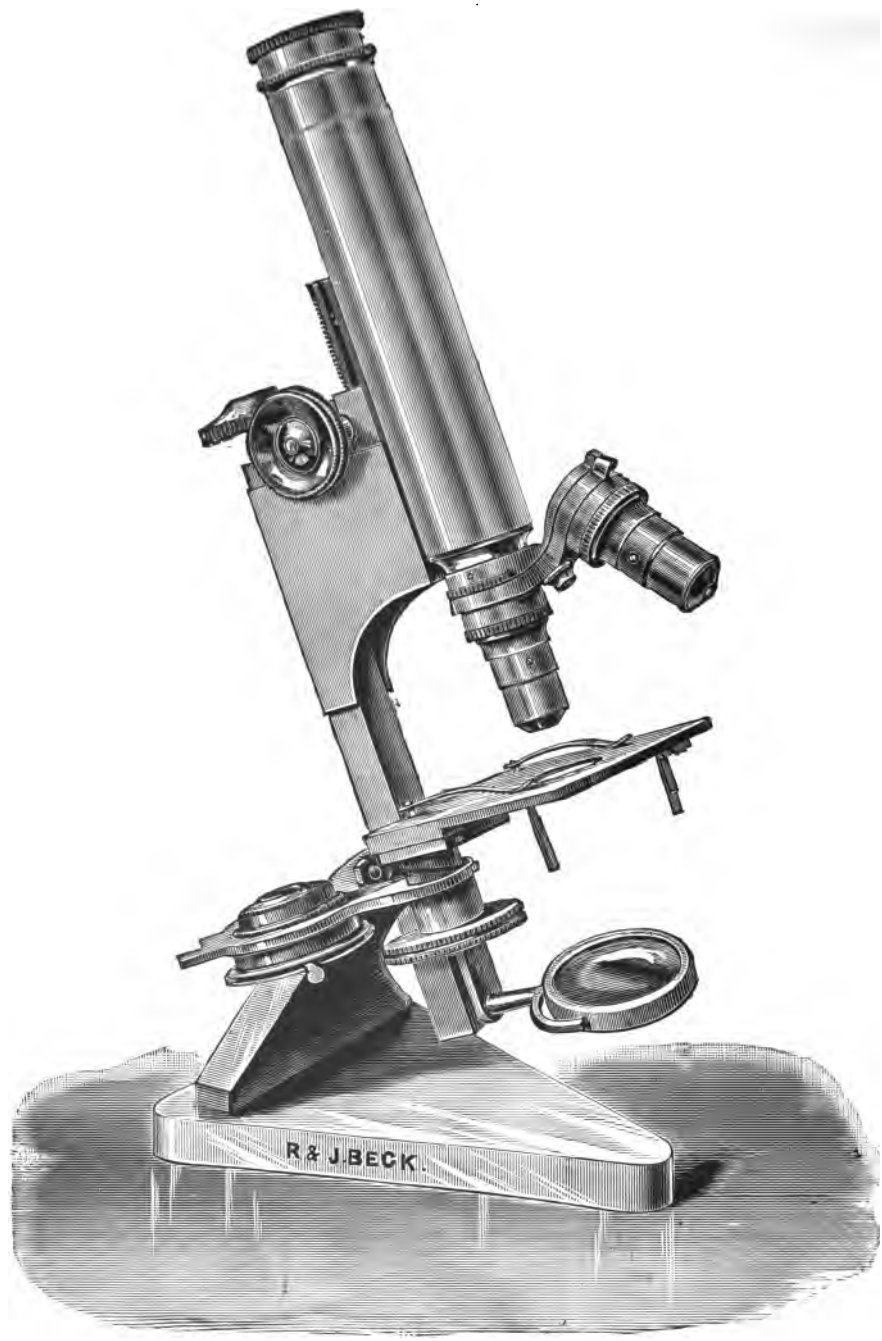

FIG. 1.

CHAPTER VIII.

MICROSCOPICAL EXAMINATION OF DIATOMS.

In this chapter the microscopical apparatus will first be dealt with, and then the methods of its employment.

The student will find that any modern cheap microscope stand made by a good English optician, will answer his purpose, provided that it be fitted with the means to focus a substage illuminator.

The most efficient of the cheaper stands is Messrs. R. & J. Beck's "Bacteriological Star Microscope." This stand possesses fine and coarse adjustments, and a simple substage fitting which can be raised or lowered (in order to focus the Abbe illuminator which it carries), or instantly turned aside. See fig. 1.

The student may rely upon any of the following maker's stands, which possess a substage :—Mr. C. Baker, Messrs. R. & J. Beck, H. Crouch, Ltd., Powell & Lealand, J. Swift & Son, and W. Watson & Sons.

If, however, a really first-class stand is required, the author has a preference for Messrs. W. Watson & Sons' "Van Heurck." The Fig. 2 gives a good representation of one of these stands.

As to objectives it is difficult to advise. A good one-sixth inch, and one inch are the most useful, and will answer most purposes, but for the most minute observations oil immersion lenses of great numerical aperture are necessary.

It is never advisable to use a higher power objective than a one-twelfth, as the amount of resolution displayed depends upon the numerical aperture, and not upon the magnifying power of the lens. Also penetration (or depth of focus, as it

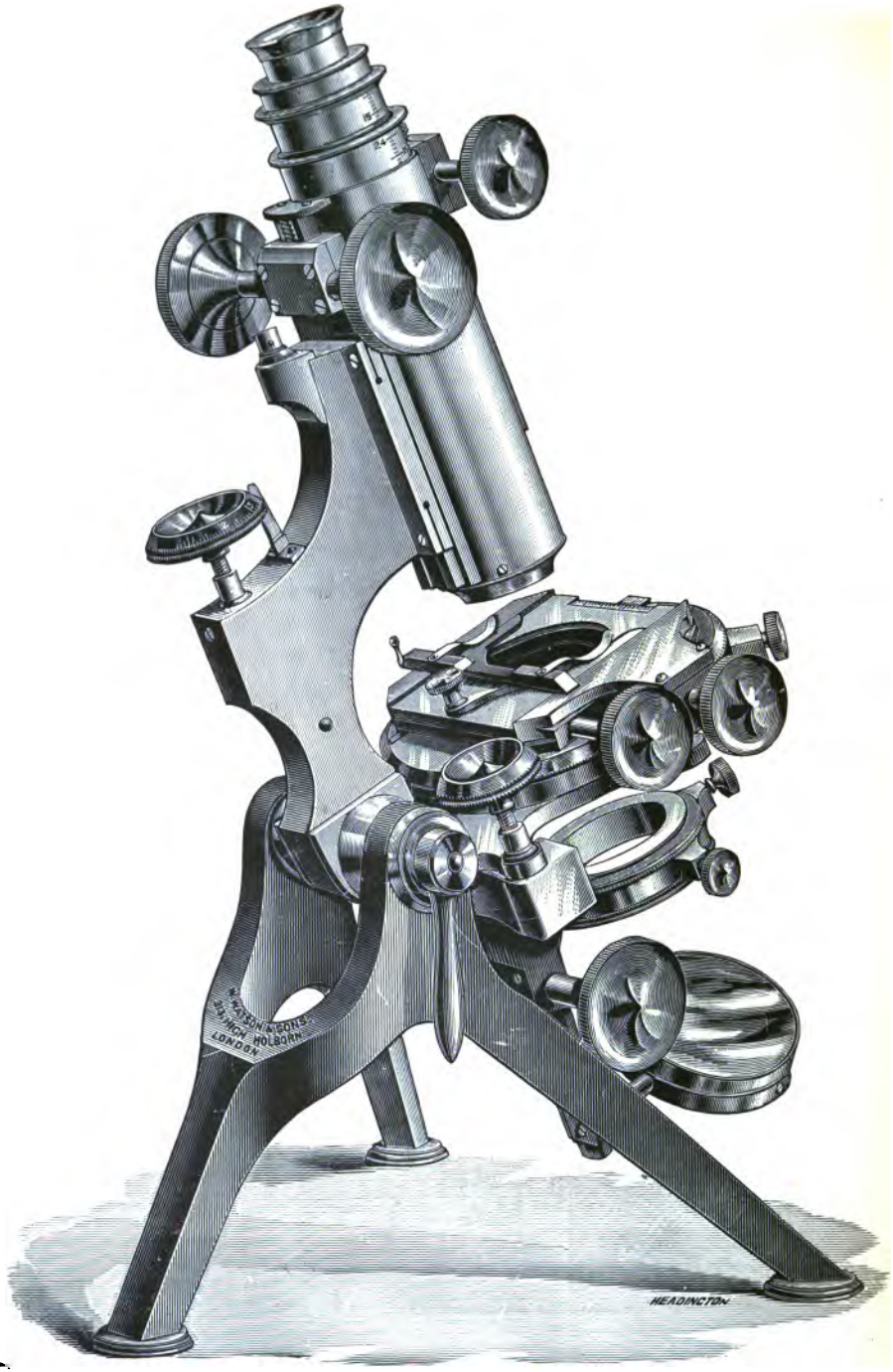

FIG. 2.

is sometimes termed) is, and mathematically must be, in the inverse ratio of the aperture, and varies at a rapidly increasing rate according to the focal length, the rate being one upon the square of the focus.

The two homogeneous oil immersions which the author prefers are Powell & Lealand's apochromatic $\frac{1}{12}$ of 1·4 numerical aperture, and Reichert's semi-apochromatic $\frac{1}{12}$ of 1·25 N.A., both of these objectives will stand a cone of light filling $\frac{3}{4}$ of their back lenses, without breaking down, the latter one giving a remarkably pure image for a cheap lens.

When working with a $\frac{1}{6}$ or $\frac{1}{12}$ inch objective a substage illuminator or some form of condenser becomes necessary. A half-inch objective screwed into an adapter fitting the substage will answer this purpose; the objective being, of course, inverted.

The ordinary Abbé illuminators, costing from 17s. to £1, are cheap and useful lenses, as they can be used with oil immersion objectives by connecting the top of the illuminator with the bottom of the slide under observation with a drop of oil.

For general work Watson's Achromatic Condenser of 1 N.A. is the most useful, as not only can it be successfully employed with lenses of large aperture, but by removing the top lens is suitable for medium powers. For the most critical work with the largest angled apochromatic objectives, Powell and Lealand's apochromatic immersion condenser of 1·4 N.A. is superior to anything that has yet been produced.

The most useful eyepiece to employ is a compensating one, magnifying about 10 or 12 diameters. The above-mentioned appliances will complete the dsciderata to enable the student to carefully examine his specimens during the day time. If work is to be done in the evening a lamp should be used. Any lamp will answer the purpose, but a microscope lamp is preferable as it enables the observer to turn the *edge* of the

flame towards the mirror. Fig. 3 shows the lamp used by the author.

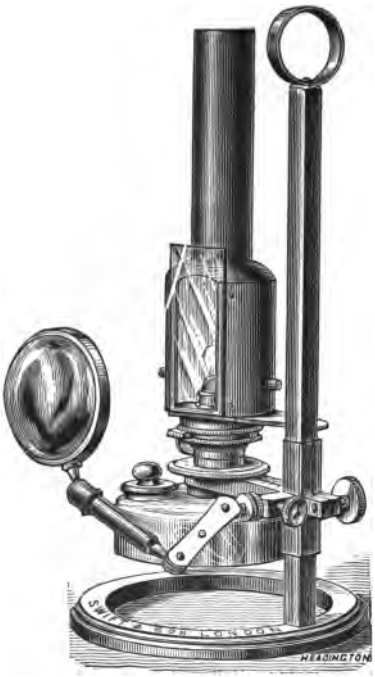

FIG. 3.

The condenser should be racked up and down until the edge of the flame is sharply focussed upon the object and by this means a critical image may be obtained.

If the objective is flooded with light a diaphragm should be placed under the condenser to reduce the angle of the cone of light entering the objective. Should this cone be too much reduced in angle a double outline will surround the Diatom.

Living specimens should be examined in a live-box, when their movements may be observed, and their velocity computed by the aid of an eye-piece micrometer, and a stop

watch. When making such examinations it is instructive to add granules of carmine (from a cake of water-colour) with a camel-hair pencil to the water containing the Diatoms. These will be seen to follow such currents as are made by the moving Diatoms, and in many instances to become attached to the gelatinous envelope, especially along the raphé of such Diatoms as possess one.

The endochrome should also be noted, particularly as to whether it takes the form of *laminæ* or of *granules*.

The yellow or brown colouring matter with which the endochrome is impregnated should be examined with a micro-spectroscope (or as it should be termed a spectro-microscope). To do this it is necessary to wash the frustules in several changes of water, in order to detach any algæ or other foreign bodies. After which the frustules should be placed upon filter paper to dry rapidly, and then immersed in a volume equal to their own of 90 per cent. alcohol and left *in the dark* to macerate, until the endochrome loses all its colour.

In about ten days the maceration will be complete and the colouring matter known as *diatomin** will be totally eliminated from the endochrome, which will be observed to be otherwise unchanged.

The alcoholic solution, after the removal of the diatomacean residue, will be in a suitable condition for spectroscopical examination.† This will prove instructive as to its consistency, particularly if comparisons be made between this solution and *Chlorophyll*.

If the beaded appearance, which is almost universal on the valves is to be examined for the purpose of ascertaining its structure the largest apertured lenses must be employed under varying conditions of illumination.

*First so-called by M. Nägeli. *Vide* Gattungen einzell. Algen, p. 7.

†The comparative spectra of *Chlorophyll*, *Phycoxanthine*, and the *Diatomin* of certain Diatomaceæ was first published by M. Petit, in Bull. Soc. Belg. Micr., vol. V., p. 55 (1880); and the chart showing these was reproduced in T. R. M. S., vol. III., pp. 680-687 (1880); and in the text of Dr. Van Heurk's "Synopsis des Diatomées de Belgique."

Dr. J. H. L. Flögel, who has probably devoted more time to systematic methods of observation on sections of valves and fractured fragments than any other investigator, has written an excellent account of his researches.

This observer recommends the following preparatory operations for enabling the investigations to be successfully carried out.*

(1) "If we wish to examine uninjured specimens. The Diatoms are first stained, usually by picro-carmine; then put in absolute alcohol. A glass slip is coated with collodion and allowed to set. To avoid peeling when dry, the collodion must not be too thick. A drop of thick gum is then put on. A cluster of Diatoms is taken direct from the alcohol with forceps and placed in the gum. In consequence of the current set up the Diatoms immediately distribute themselves equally through the gum. As soon as the edge of the gum begins to harden, one frustule after another is drawn to the edge by a very fine needle, where with proper manipulation, they can be piled up like a bundle of rods. All those which interfere with this piling up should be removed. Owing to the staining, the frustules can be readily seen on the transparent ground. As soon as the edge dries, a drop of fluid gum is added by a needle, and this process is repeated until the solid layer of gum has such a thickness that a displacement of the frustules need be no longer feared; a small patch of collodion is then put on. The preparation is now cut out by four cross cuts and carefully removed from the glass; the bundle of Diatoms in gum being contained between two films of collodion. It is advisable to make a drawing with a high power to show the position of the individual frustules and aid in the identification of the sections. The preparation is then put upon a nearly dry flat drop of gum on a piece of cardboard, and the base and edges are made to adhere, if necessary, by a few drops of water, and by the addition of minute drops of gum it is so imbedded that at last it is

* *Vide* Bibliography.

entirely surrounded. This must be carried out so cautiously that the collodion films do not separate; very careful watching of the imbedding process is therefore necessary, especially also to avoid cracks which commence at the edge and might easily extend to the object. From this bundle sections may be made according to the method described by me in 1870.

(2) "Anyone wishing to study the structure of the individual valves and mark their manner of combination can shorten this somewhat detailed process. A cluster of Diatom valves is taken out of the alcohol and placed in a large drop of water on a slide; and the water is allowed to evaporate after the valves have been evenly distributed. A drop of gum already dry—if possible with a flat surface on a piece of cardboard—should be in readiness; another piece of glass is coated with oil of turpentine, which is allowed to run off so that a very thin film is left, which does not readily dry. In this the point of a fine needle is dipped vertically, taking up sufficient oil so that by touching a frustule lying on another slide it will adhere. Thus the frustule can be put on the hardened drop of gum which has been moistened by the breath; this is repeated with a number of valves *ad libitum*, and finally they are covered with minute drops of gum till the required thickness is attained. This transference of dry frustules upon the dry gum is much easier than the process with fluid gum described under (1), because, with the latter it frequently occurs that in bringing a new frustule into place the others are disturbed. With uninjured frustules, process (2) is not available, because these, after the drying of the thin upper layer of gum, moistened with the breath, will at once become charged with air and baffle any cutting. This absorption of air can only be avoided by transferring the frustules from the alcohol directly into the fluid gum, which then diffuses equally through them. At the most the frustule at the moment of hardening is slightly compressed, which injures somewhat the appearance of the section."

FIG. 4.

CHAPTER IX.

How to Photograph Diatoms.

To photograph Diatoms a camera specially made for that purpose will be required. This may either be vertical as shown in Fig. 4 (as used by the author) or horizontal as in Fig. 5. The engravings clearly show the construction of these cameras. Whether one or the other of them be used it should be furnished with two focussing screens, one of ground glass as usual, the other of plain plate glass ; the former being suited for roughly getting an idea of the general contour of the specimen, the latter is used merely as a support for a focussing eye-piece used for focussing the aerial image. This eye-piece is adjusted so as to be in focus for a small object placed under the opposite side of the plate glass when the eye-piece is held in contact with the other surface of the glass. Just in front of the focussing screen a groove should be cut, permitting of tin plates to be used as masks to give a clean edge to the photograph. The most suitable size of camera for the above apparatus would be half-plate, $6\frac{1}{2} \times 4\frac{3}{4}$ inches.

The microscope tube should be of large diameter, unless a projection ocular is to be used; its length also is an important matter, but most opticians are now making microscope stands with a series of draw tubes, which enable one to vary the tube from 6in. to 12in., and to work at any length that seems to give the best results.

It is important to see that the inside of the microscope tube is uniformly of a dead black.

It is essential that the objectives should be corrected for photography, that is to say, the visual and chemically actinic foci must be coincident, or the photographic results will be

blurred and out of focus. Most opticians supply suitable objectives.

Very good work can be done without an eye-piece, but the

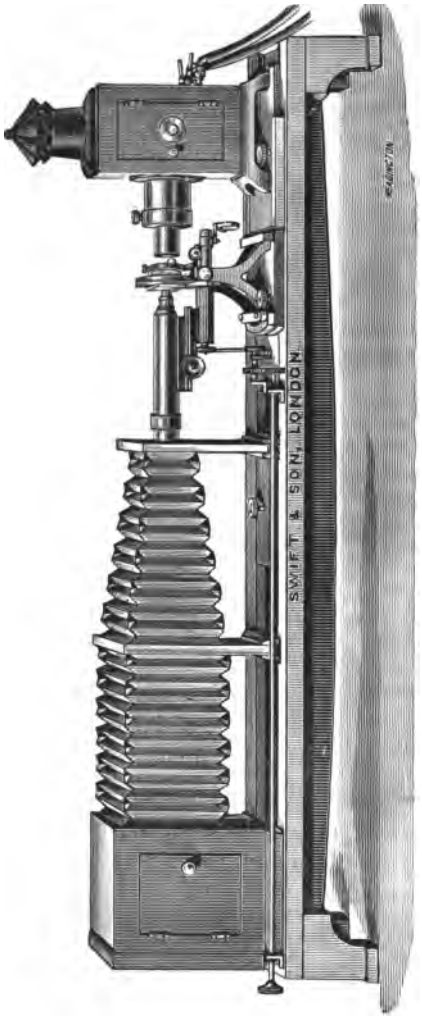

FIG. 5.

very best can only be accomplished with its aid. It must be clearly understood that the ordinary eye-piece is not adapted

to photography, and that only those constructed specially for the purpose must be used. These are fitted with one end rotating in a spiral slot, the use of which is explained subsequently.

Undoubtedly, a projection ocular is a great convenience, as the camera does not require to be so long, 30in. being ample, and at least two-thirds less than the extension generally required when working without one.

It is worthy of note that Zeiss's low power *compensating* oculars may be used for projection in place of his projection oculars, with excellent results.

The Dark Room.

Although development may be carried out in the bedroom at night, in a cupboard, under stairs, in a wine-cellar, and other similar places, they are obviously inconvenient and quite unsuitable for systematic scientific work. A room should be fitted up especially for the purpose, and possibly the most convenient course will be to have means of converting the room in which the apparatus stands into the dark room—in other words contrive that the dark room shall be of such size that the apparatus can be kept *in situ* in it.

The room should be well ventilated, this being a very important item, as when artificial light is used the air gets very foul, especially when ammonia is being used. A good water supply, and a sink, over which development can be carried out complete the essential requirements.

The Illumination.—Gas is the most convenient when it can be obtained. One jet being provided with a by-pass, the pilot flame of which burns behind a collar, thus showing no light when turned down; this serves to illuminate the room on ordinary occasions, and for exposing bromide prints, &c. Another jet is placed inside a lantern glazed with ruby glass:

the screen holding the glass being removable and replaceable with one of yellow, for use during printing, or the development of plates other than orthochromatic. If gas is not available, a paraffin lamp or candles may be employed but these are not so generally useful as gas, the heat from gas being easily adapted as a means of ventilating the room. The flue from the lantern should be led out of the room.

A set of about half-a-dozen dishes of porcelain will be found sufficient. Two or three shelves ought to be fitted up to hold the bottles of reagents. The reagents required are as follows, and the following strengths will be found convenient for photographic use :—

Pyrogallic acid*	10%
Bromide potassium	10%
Ammonia	10%
Potass. ferrocyanide	10%
Quinol	crystalline
Potass. hydrate	in sticks
Potass. metabisulphite	powdered
Sulphite of soda	crystalline
Citric acid	crystalline
Ferrous sulphate	sat. sol.
Neut. oxalate potass.	sat. sol.
Mercury percnloride	sat. sol.
Thiosulphate soda (hypo)	sat. sol.
Powdered Alum	sat. sol.

The first three substances on the list are most conveniently kept in dropping bottles of about two to four ounce capacity. The alum and hypo being kept in stone jars.

Two measuring glasses should be obtained, one of ten-ounce capacity and another of a drachm. A pair of small balances with glass pans are also required.

The fixing bath should have a wooden lid to prevent dust falling in, it also prevents evaporation when not in use.

A wooden grid should be fixed above the sink to develop upon, this arrangement letting the spillings, &c., fall into the sink below.

The Illuminant. This may be either—

(*a*) Oxy-hydrogen lime light.
(*b*) Coal gas.
(*c*) Paraffin.
(*d*) Daylight.

(*a*) The oxy-hydrogen light is probably the most convenient for the majority of workers, two forms of jet being available—

The "blow-through."
The mixing jet.

The former is the safest for beginners to work with, the oxygen gas being blown through a hydrogen flame upon the lime cylinder, but the light is not so good as that obtained from the latter, in which the oxygen and hydrogen are mixed in a chamber previous to consumption at the nozzle. The nozzle of this jet should be fine in bore, rather finer than when for use in the lantern ; by this means a nearer approach is obtained to the theoretical "point of light." A very good mixing jet, invented by Mr. Andrew Pringle (made by Messrs. Newton and Co., of Fleet Street, London), is provided with a special cut-off tap, which in one movement cuts the oxygen off completely, and turns at the same time the hydrogen down to a minimum, as shown in Fig. 6.

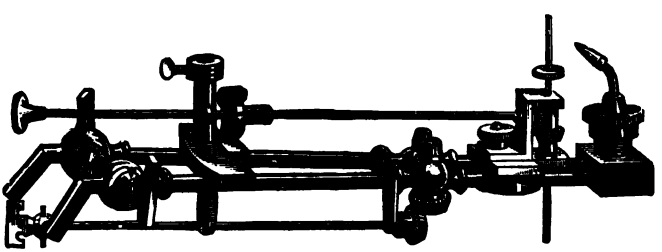

FIG. 6.

The limes should be of the hardest description ; Nottingham limes being the best we have tried. All good jets are provided with an arrangement for turning the limes when they become pitted, for if their use be continued in this state, the flame is liable to spurt sideways, and so crack the condenser. The gas for the jet can be obtained compressed in steel cylinders. The author uses the oxygen from a cylinder provided with a Beard's regulator which renders possible the adjustment of flame at the taps of the burner ; but he obtains the hydrogen from the mains—that is he uses coal gas for hydrogen. The only danger in this method of working is that unless the oxygen be turned on carefully, it may be blown round into the mains and not only extinguish the light at the jet, but furnish an unpleasant surprise to the next person who attempts to light the gas in another part of the house. With care however this will not happen.

(*b*) Coal gas alone, may be employed with an Argand burner with fair success, but a Welsbach flame, (which is a Bunsen flame playing upon the interior of a cap made of cotton fibre netted and coated with a mixture of metallic salts, chiefly oxide of zirconium) acts better.

(*c*) An ordinary paraffin microscope lamp is also available, but is better suited to low powers than to high.

(*d*) Daylight requires special arrangements, scarcely within the reach of ordinary workers ; a heliostat is needed to keep the sunlight upon the condensers.

Monochromatic Light.

Fluid saturators in cells, or coloured glasses placed between the condenser and the light are of little, if any, service in improving the photographic image. Mr. E. M. Nelson's apparatus described in J. R. M. S., 1891, p. 443-6, answers very well and with regard to resolving power with a blue-green light, it practically adds ·1 to the N. A. of the objective ; thus a D.D. of ·8 becomes a lens of ·9 N. A., and

without increasing the sperical aberration. There can be no secondary spectrum with monochromatic light, therefore an ordinary achromatic lens acts as well as an apochromatic.*

Exposure of the Plate.

First go into the dark room, shut the door, and light the red lamp, then open a box of plates, and one of your double dark slides. Take two plates out of the box, dust the film side carefully with a broad, soft brush, and place them in the dark slide, the film side of the plates being turned towards the shutters of the double dark slide. Carefully fasten up the remainder of your plates in the box, and daylight may then be permitted in the room. Screw on to the body a one inch power, and place the microscope ready to attach to the camera. The light, whether oil lamp, electric, or any other, must be placed in the exact optical axis of the microscope, together with the condenser. Focus both the objective and the condenser upon the object placed on the stage. Too much stress cannot be laid upon the importance of having the condenser perfectly centred, whether it be the large four-inch condenser, a wide-angle substage condenser, or an ordinary large bull's-eye. The image of the flame must be exactly focussed in the centre of the field. If it is too much on one side, the opposite edge of the field will be dark.

Presuming that all is ready, roughly focus with the ordinary eye-piece and then remove it, inserting in its place a projection eye-piece. Bring the camera into position, but so that the fingers may grasp the revolving head of the eye-piece, which must be turned backwards and forwards until the round disc of light thrown upon the focussing screen has a sharp and clearly defined margin. The camera can now be connected with the microscope.

**Vide* J. R. M. S., 1891, p. 442; 1892, p. 1.

Withdraw the shutter, uncovering the plate, and after taking care that the camera no longer vibrates, remove the black card from the lamp for, say, one minute, with an ordinary oil lamp having a $\frac{1}{8}$in. wick or about twenty seconds with lime-light. The shutter having been replaced and the slide removed, the plate should be developed as described later on.

Development.

In the last chapter we have seen how the plate is to be exposed. Our attention must now be turned to the process of development, by which the latent image in the film is to be brought to light, thereby making a negative of the plate from which any number of positive copies may be printed.

First, the developing and fixing solution must be prepared. There are several reagents which act as developers of the latent image. That which is still most generally used is pyro and ammonia, or alkaline pyro. This should be prepared in three stock solutions, viz :—

No. 1.

Pyrogallic acid	1 ounce.
Sulphite of soda	3 ounces.
Water to make	10 ounces.
Sulph. acid	5 drops.

The sulphite (take care it is pure) must *first* be dissolved in hot water, and when cold the pyro added to the solution, or the pyro ready made up in a 10 per cent. solution may be obtained at a photographic dealer's under the name of sulpho-pyrogalol.

No. 2.

Bromide of potassium	1 ounce.
Water to make	10 ounces.

No. 3.

Liquid ammonia ·880	1 ounce.
Water to make	10 ounces.

These will each be, roughly, 10 per cent. solutions, consequently, any proportion of the three may be measured out, 10 drops of any one of the solutions containing one grain of the substance dissolved or of ammonia.

What may be called a normal developer, that is for correct exposures, may be formed by taking of

No. 1	20 minims	to each ounce of water.
No. 2	10 ,,	
No. 3	20 ,,	

Another developer in common use is hydroquinone or quinol, of which the following is a formula :—

A.	Hydroquinone	3 drachms.
	Potassium bromide	40 grains.
	Sulphite of soda..	3 ounces.
	Water to make	20 ounces.
B.	Caustic potash	2 drachms.
	Water to make	20 ounces.

Equal parts of A and B are used to develop. This developer has the advantage of not staining the fingers (as does pyro), and may be used to develop several plates in succession.

A third developer which has much to recommend it is eikonogen. This can be bought in a very handy form of cartridges. The contents of a cartridge are dissolved in about six to eight ounces of water, and the developer is at once ready. This has the advantage of saving trouble. It leaves no scope for varying the constituents of the developer to suit special cases, but that is, perhaps, of less importance in photo-micrographic work where the exposure may be repeated to suit the developer used. The same eikonogen developer may be used for many plates in succession, but gradually loses its power.

One of the above-mentioned developers having been chosen (we recommend the latter for beginners, but personally prefer

pyro), the fixing bath must also be mixed and placed ready for use in a tray *kept exclusively for that purpose.*

The strength of the bath should be about—

Hyposulphite of soda	1 ounce.
Water	6 ounces.

A rough idea of the object of the developing and fixing solutions may be given as follows :—Wherever light has acted on the plate through the objective forming the image, there the developer causes a visible reduction of silver as a black deposit, and the intensity of this deposit varies according to the light which that part of the plate has received. Dark portions in object will, therefore, remain white or creamy, and the highest lights of the object will, in the negative, be black. The intermediate tones will be represented accordingly, and so a representation reversed in lights and shades, or, as it is called, a negative is produced.

The fixing solution: Hypo has the peculiar property of dissolving out all the salt of silver (that part which was not acted upon by light) in the film, and has no power of affecting the reduced image or black portions. Consequently, the shadow portions are, after fixing, more or less clear glass.

Let the beginner remember that the aim in photo-micrography is to expose and develop in such a manner as to obtain a negative which shall give in the subsequent print all the detail of the original and shall show, as far as possible, the same gradations of light and intensity of colour. To approach correct relations of colour the photo-microscopist must have used, in exposure, orthochromatic plates and coloured screens (as we have already pointed out) and in such a case only rather *deep red light* may be used when the plates are taken out of the slides and developed. If ordinary plates have been used, orange light is to be preferred as being quite safe, and as giving a much brighter light by which to see the development. Ruby paper or orange paper form excellent media.

Now, as to the actual operations and judgment needed in developing. Having the fixing solution to hand in one tray, remove the exposed plate from the dark slide, and place the film (the duller side uppermost) in the other tray. Now pour the developer on it in such a way as to cover the whole plate quickly and evenly. Rock the plate gently so as to keep the developer moving; otherwise, fatal markings with some developers may be caused in the negative. If the subject have been correctly exposed, the image will shortly appear, the high lights of the image coming first, and the whole gradually growing in detail and density. Now comes the necessity for experience and judgment, though there is no reason why the very first plate developed by a beginner may not be one of the best. The difficulty is to decide whether the plate has been over or under exposed, and then, more important still, to know at what stage to stop the development. Now, if the normal developer be used and the temperature always kept the same, the time that the image takes in appearing is some criterion of the exposure. If over-exposed the image comes quickly, if under-exposed it is longer in appearing. But the better way of judging is to watch the character, rather than the time of appearance, of the image. If black patches appear in certain parts, the rest remaining white, then *under-exposure* is to be feared, and a plate much under-exposed is useless to the microscopist. If the plate shows a tendency to *grey all over* then the subject has had *too much exposure*, and immediate steps should be taken to make the best of such state of things. These we shall indicate immediately. Judging by surface appearances, however, is somewhat misleading with some makes of plates, and, as far as ultimate density is concerned, practically useless. The only safe guide is to examine the the plate by transmitted light, holding it up to the red or orange light. The same rule holds good here. If the plate comes black in patches it has been under-exposed, if thin and grey all over it has been over-exposed; some allowances, of

course, being made for what may have been the actual character of the contrasts in the object photographed. The plate which comes rather grey all over and thin at first need never be despaired of. It must be remembered it is the print that has to be considered, not the mere appearance of the negative. These are golden rules here to be born in mind. If the plate has evidently been under-exposed, and comes harsh in contrasts it should be further developed, or else the subsequent printing process will not give even a presentable print. If the plate shows evidences of over-exposure, the development must be prolonged until the image is very dense—until, in fact, very little sign of image can be seen in the blackness. In such a case, if good thick emulsion plates have been used, an excellent, though slow, printing negative may still be obtained. This is the great difficulty in developing—to know when to end it. A few experiments with the dark room, the plates, and the printing method which the beginner may have chosen to use, will soon give him a practical of his work.

It is usual to vary the constituents with the pyro-ammonia developer, if the plate comes up as if under-exposed, the normal developer is poured off and washed out, and one containing, say, 1 grain of pyro, ¼ or ½ grain of bromide, and 20 drops of 10% ammonia *per ounce* of developer is applied. Even more ammonia may be added if the plates seem able to stand it. If the plate shows over-exposure, the normal developer is discarded as before, and one containing, say, 4 grains pyro, 2 of bromide, and 1 drop of ammonia per ounce is employed, and the result patiently waited for, as it will come very slowly. Above all, let it be remembered to develop over-exposed plates to very full density, for good printing purposes.

When development is considered complete the plate is well rinsed, and, if thought desirable, cleared of pyro stain by immersion in an alum bath (saturated solution) for two

or three minutes. Alum, however is not altogether desirable, and if the plates have no tendency to frill, a weak citric acid clearer (1 in 80) is better. After the alum or acid the plate is again well rinsed, and is then placed in the fixing bath for about ten or fifteen minutes. The plate may be examined from the back, and if every vestige of the white appearance is gone a few more minutes will complete the fixation. The hypo bath may be used for many plates, but it is not advisable to allow it to get very discoloured or overcharged with silver. The plate is now to be very thoroughly washed. This may be done in daylight, and if in running water, one hour or even less, is sufficient; or six or seven changes of water, made at intervals of about ten minutes, will be found to suffice. The plate, after washing, should be cleaned at the back (the glass side) and placed in a rack, or leaning against a support, to dry naturally. When dry it is ready for printing, or it may be varnished as a protection against the possible danger of damp silver paper, which causes red stains in the film, almost impossible to remove.

Should the plate prove too thin, it may be intensified. This may be done by placing it in a 1 in 20 solution of mercuric chloride. This will bleach the film, and the bleaching should be allowed to act right through the film. The plate must then be thoroughly washed for, say, five or ten minutes, under running water, and placed in a bath of dilute ammonia (1 in 20) until it turns quite dark all over. Finally the plate must be washed, and can then be placed on the rack to dry.

Printing.

The negative having been obtained and its defects, if any, remedied, the next process is its printing, and for this purpose, to our minds, none is more beautiful than carbon, which has this advantage, that it is absolutely permanent, and the disadvantage which is usually urged, namely the

reversed position of the image, does not apply to photo-micrography. We shall describe this process only, because by means of it we can both make prints and lantern slides, the latter being in the majority of cases the means taken for displaying the specimens for public demonstration; an additional advantage being that by means of this process the final image can be obtained of any colour, thus rendering the specimen a nearer approximation in many cases to the original specimen. The first step is to put what is termed a safe-edge round the negative, this being most conveniently done by pasting four strips of non-actinic (black, or red) paper on the back of the negative, so as, as it were, to make a complete frame round the image. The reason for this frame is that without it the edges of the carbon tissue would wash up during the subsequent development.

The negative is now placed in the printing frame and a piece of sensitised carbon tissue* laid upon it. The exposure of this tissue must be judged by means of an actinometer as the image is practically invisible. Several forms of actinometers are made for this purpose, consisting of a graduated scale of tints superimposed on a strip of sensitised albumen paper. A trial print is made in silver and the actinometer is exposed simultaneously, and when the silver print is correctly printed the number of the actinometer square, which has *just become visible*, is noted. This forms a guide for future exposures, it being only necessary to watch the actinometer. As the tissue gets older it requires slightly longer exposure; it remains in good condition for about ten days after sensitising. The print having been removed from the frame, the next step is development, which, in the case of photo-micrographs can be done on the final support at once. If the specimens are to be shown at public meetings, a very good way is to develop them upon sheets of matt surface

* The Autotype Company send out Carbon tissue freshly sensitised two or three times a week, thus saving a good deal of trouble to amateurs in sensitising the tissue themselves.

opal glass, thus giving very brilliant results; but if the specimens are for preservation in books, they can be developed upon *autotype single transfer paper*, or if for lantern slides, upon glasses 3¼ inches square (which, after being first thoroughly cleaned), are coated with a substratum of gelatine by immersing them in a bath of

Gelatine	1 ounce.
Water	10 ounces.

and subsequent drying and immersion in

Pot. bichromate	2 drachms.
Water	10 ounces.

And exposure to day-light for an hour.

Whatever may be the substance of the final support, the treatment of the tissue is the same. It is immersed in a dish of cold water, and is left there until the tissue, which at first curls, begins to uncurl. The surface is then brought into contact with the support under water, and is firmly squeegeed to force into accurate contact and expel all air-bubbles. The support and tissue, now in contact, are placed to partially dry between sheets of blotting paper under pressure. After about 15 minutes they are removed and developed by immersion in warm water (*Fah.* 100°) until the pigmented gelatine is seen to ooze from the sides of the paper, which is now raised by the corner and pulled away, and the water kept moving over the surface of the print, either by shaking the dish from side to side, or by laving warm water on to the surface with the hand, the bathing in warm water being continued until the high-lights are quite clean and clear. To secure this, the water is changed two or three times, and the last bath had better be slightly warmer than its predecessors (*say Fah.* 120°). The print is now immersed in alum solution 5%, and left there to harden for about 20 minutes, when it is rinsed in water and dried.

The process takes longer to describe than to do, and it is quite easy to manipulate.

It is better for photo-micrographs to have a glazed surface as this tends to show the detail better than when the matt surface is retained. The prints can have a high glaze placed on them by first hardening the gelatine by immersion in alum 5% solution, then squeegeeing into contact with sheets of carefully cleaned *polished* plate glass—or specially enamelled iron plates sold for this purpose (these have the advantage of not needing such careful cleaning as the glasses, although the surface thus given is not so highly polished). When dry the prints strip off of their own accord.

If the prints are to be mounted on cards, and the polished surface retained, they should have a thin line of stiff glue applied round their edges to a depth of about $\frac{1}{16}$ of an inch (applied by means of a brush and straight-edge) and the print pressed into contact with a *warm* flat-iron. If starch is used as a mountant the water from the starch would soak into the gelatine and so destroy the gloss before the print could be placed on the mount. If starch must be used it may either be applied to the print whilst it is still damp on the plate glass or enamelled iron, and then allowed to dry, and the card *only* damped for mounting purposes, or as an alternative process, when the prints are all on the enamelling plate paste them all over together whilst still damp, and place a sheet of fine thin paper over the whole of them, and peel them all off at once, when dry. They can then be trimmed and mounted in the usual way, the extra thickness of paper preventing the gelatine surface receiving any moisture through the back whilst they are being affixed to their mounts.

As lantern slides must often of necessity be made at night, it will be as well to give a rough sketch of gelatine-bromide lantern plate manipulation. The size of a photo-micrograph renders it possible, in the majority of cases, to make lantern slides from them by means of contact. With average negatives and average lantern plates, an exposure of 15 to 20 seconds to gaslight of a fish-tail burner, at a distance of

about one foot from the flame, will be found ample. Development is best carried out by means of some form of Quinol developer. The following can be recommended:—

A.
Quinol	80 grs.
Sodium sulphite	3 oz.
Pot. bromide	20 grs.
Metabisulphite potassium	1 drachm.
Water	10 oz.

B.
Potassium hydrate	60 grs.
Water	10 oz.

Mix the above solutions, A and B, in equal parts, do not wet the plate before development, as this often causes the formation of pinholes in the film. The above developer will be found to give sufficient density, and will retain the high lights absolutely pure. Development should not be carried quite so far as is desired in the finished result, as during the pouring off of the developer a wonderful increase in density takes place. The plate is now fixed in a solution of

Sodium thiosulphate (hypo)	4 oz.
Water	20 oz.

and after having thoroughly cleared it is washed as if a negative. Should the plate be too dense it can be reduced by immersion in

10% Solution ferrocyanide of pot.: (*yellow* prussiate)	30 minims.
Sodium thiosulphate solution (as above)	1 dram.
Water	1 oz.

care being taken that too much reduction does not take place.

The slide having been allowed to dry, two little discs of paper (gummed stamp edging) are affixed, one in each upper corner on the gelatine surface of the plate, and the surface is then covered with a piece of clean glass the same size as the plate, and the two are bound together with strips of gummed paper. The object of the discs of paper is to show which way the specimen is to be inserted in the lantern during exhibition, the discs being placed downwards and towards the condenser.

CHAPTER X.

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